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GROWTH OF RATS FED HIGH PROTEIN RATIONS SUPPLEMENTED BY DIFFERENT AMOUNTS AND COMBINATIONS OF VITAMINS B (B_1), G (B_2), AND B COMPLEX*†

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WHEREAS it is generally conceded that a ration rich in protein is adequate for carnivora, for many years investigators have expressed their conviction that omnivora and herbivora suffer in some manner from such a regimen. Besides attributing to high protein dietaries a deleterious effect upon renal and vascular tissue, some workers claim that restricting an animal to such food during the period of rapid development results in a retardation of the rate of growth. Reports, however, are conflicting. A survey of the literature on the subject indicates that both normal and subnormal rates of growth are recorded for rats subsisting on rations containing 45 to 95 per cent of casein.

An opinion has also been expressed that a diet having a high protein constituent increases the animal's need for B vitamins (1, 2). Sherman and Gloy (3) investigated this subject by employing rations containing 12 to 54 per cent of casein supplemented with orange juice as the source of vitamin B complex. Within the limits of their experiment, these authors found no basis for the belief that the rat's need for vitamin B complex is influenced by the proportion of protein in the food consumed.

A retarding effect upon the rate of growth of rats as the protein constituent of the ration increased beyond 14 per cent, has been reported by

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Slonaker (4). In the mixture of foods which he used, vitamins of the B group were contributed chiefly by 1.9 per cent of yeast and 2.8 per cent of wheat germ; without doubt these vitamins were contained also in other dietary constituents such as whole wheat, whole corn, and commercial skimmed milk powder. To increase the protein content above the lowest level (10.3 per cent) which this ration supplied, Slonaker states that meat scrap was added to it in such amounts as to increase its protein component to 14.2, 18.2, 22.2, and 26.3 per cent respectively. As no mention is made of the addition of yeast or of some other potent carrier of the B vitamins to these four diets, it is assumed that such adjuvants as were contained in the unmodified food must have been diluted progressively as meat scrap was added in increasing amounts.

The best rate of growth is reported for rats maintained on the ration which contained 14.2 per cent of protein. In the diet which furnished the highest level of protein used in Slonaker's investigation, the yeast and wheat germ components evidently had been reduced to 1.2 and 1.9 per cent respectively. He states that half of the females restricted to this latter dietary were sterile. Such an incidence of sterility as well as a retarded rate of growth due to the ingestion of protein in this or even higher quantities, are findings which have not been confirmed by other laboratories. Possibly incipient dietary deficiencies were responsible for the retarded growth and the abnormalities which Slonaker observed.

The data here presented were collected during the progress of another investigation (5) for which most of the rats had been subjected to a right unilateral nephrectomy. That this procedure, *per se*, does not interfere with the growth of the rat has been shown by Smith and Jones (6). Moreover, each experimental nephrectomized rat restricted to the food containing either 50 or 90 per cent of casein, is compared with a nephrectomized rat maintained on a control diet. In addition to this, other groups with both kidneys intact, have been fed both the high-protein and the control diets.

Experimental Procedure

Female albino rats were used in this investigation which covered a period of 56 days. The fourteen groups which were nephrectomized were operated upon at the beginning of the experiment when 30 days of age. Four groups of intact animals also were used.

Diets containing 18, 50 and 90 per cent of casein (Table I) were fed *ad libitum* and vitamin-bearing adjuvants were supplied apart from the ration six times a week. Each rat received a daily allowance of four drops of

TABLE I
COMPOSITION OF EXPERIMENTAL DIETS

Rat Groups	A	P	Z
	%	%	%
Casein*	18	50	90
Dextrin†	76	44	4
Agar‡	2	2	2
Salt Mixture§	4	4	4

* Casein no. 453—Casein Mfg. Co., Bainbridge, N. Y.
† Commercial Dextrin, white. Eimer and Amend, N. Y.
‡ Agar U.S.P. coarse powder—Merck & Co., New York.
§ Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 919, 37, 557

cod liver oil (116 mgm.) and two drops of wheat germ oil[1] (43 to 45 mgm.).

All the groups of rats which ate a ration having 18 per cent of protein are designated by the letter A; similarly, P indicates those groups restricted

TABLE II
DIETARY SIGNIFICANCE OF THE NAMES OF THE GROUPS OF RATS

Group names designating casein constituent of diet			Amounts and combinations of vitamins B, G and B complex represented by numbers 1 to 7 in the group names
% 18	% 50	% 90	
A1	P1	Z1	0.4 gm. of yeast
A2	P2	Z2	0.8 gm. of yeast
A3	P3	Z3	1.6 gm. of yeast
A4		Z4	1.6 gm. of yeast plus 3 drops (88.5 mgms.) of tikitiki extract daily.
A5		Z5	1.6 gm. of yeast plus autoclaved yeast mixed into the ration at a 7 per cent level until consumption reached 10 gms. of food daily, then the quantity was reduced to a 5 per cent level.
A6		Z6	0.8 gm. of autoclaved yeast plus tikitiki extract. 1 drop (27.5 mgms.) was given for the first 22 days, the amount was then increased to 9 drops weekly, then to 2 drops daily and finally to 3 drops daily.
A7		Z7	5 drops of tikitiki extract (147.5 mgms.) were fed during the first 46 days, the drops were then increased to 6 and finally to 12 daily. On the 28th day of the experiment and thereafter, 0.4 gm. of autoclaved yeast was given daily.

[1] Kindly furnished through the courtesy of Mr. M. W. Tapley of E. R. Squibb & Sons, Brooklyn, New York. Wheat germ oil no. 665 AH.

to the food containing 50 and Z, 90 per cent of casein. The letter K preceding A or Z indicates that all rats in groups so designated, were animals having two kidneys.

The amounts and combinations of vitamins B (B_1), G (B_2), and B complex supplied to each rat are indicated in the group names by numbers (Table II). These adjuvants were furnished by dried yeast,[2] tikitiki extract,[3] and autoclaved yeast, and were fed six times a week apart from the food mixture, except as specified to the contrary.

Discussion of Results

The level of dietary protein is only one of many factors (7) which may stimulate or depress the rate of growth of rats. In addition there are doubtless uncontrollable conditions which influence growth; even in paired feeding experiments, Mitchell and Carman (8) have observed that the gain in weight of rats is subject to variation because of the influence of what these authors term "unknown factors." Nevertheless, we feel that the importance of such unknowns is likely to be greatly minimized in the interpretation of our data, because of the numbers of rats represented in each group (Table III).

The mean gains in weight shown by groups of nephrectomized rats are not significantly different from the gains of intact groups restricted to the same ration (Table IV), even though the food calories consumed by 50 per cent of the groups which had two kidneys, are significantly higher than the caloric consumption of corresponding A and Z groups. This condition indicates that the nephrectomized rats have utilized food for growth better than have the rats possessing two kidneys. It is striking that this similarity in growth between the two groups should be maintained in the face of an excessive amount of dietary protein.

An adequate food mixture containing 90 per cent of casein and supplemented with 0.4 gm. of yeast produces inferior growth in both nephrectomized (Z1) and intact (KZ1) rats when they are compared with their respective controls (A1 and KA1) (Table V), as indicated by the significance ratios. However, there is no significant difference between the gains in weight of the Z1 and KZ1 groups (Table IV).

When the yeast allowance was increased four times, the intact rats (KZ3) grew as well as their controls (KA3) but the nephrectomized rats (Z3) made significantly inferior gains in weight in comparison with their

[2] The yeast was obtained from the Northwestern Yeast Company, Chicago.

[3] Kindly furnished through the courtesy of Dr. Brown, Philippine Bureau of Science, Manila, P. I.

TABLE III

MEAN GAINS IN WEIGHT AND FOOD INGESTED BY GROUPS OF RATS MAINTAINED FOR 56 DAYS ON RATIONS CONTAINING 18, 50, AND 90 PER CENT OF CASEIN SUPPLEMENTED BY DIFFERENT AMOUNTS OF VITAMINS B, G, AND B COMPLEX

Group	Mean gain in weight	Probable error	Significance ratio	Food calories ingested*				Number of rats
				Protein	Total	Probable error	Significance ratio	
	gms.							
A2	143	±3.55		454	2483	±46		14
A4	129	±4.85	2.33	527	2678	±81	2.1	12
A3	129	±2.97	3.02	480	2414	±41	1.1	20
A1	128	±2.34	3.53	399	2308	±51	2.5	14
A5	112	±3.25	6.44	496	2358	±45	1.9	13
A7	108	±2.27	8.31	374	2293	±28	3.5	12
A6	83	±3.95	11.30	350	1888	±57	8.1	12
P2	135	±3.43		939	2222	±12		9
P3	133	±4.52	0.06	998	2338	±28	3.7	9
P1	128	±3.39	0.29	878	2091	±19	5.7	9
Z4	118	±5.87		1672	2175	±52		12
Z5	114	±3.45	0.58	1690	2238	±54	0.8	12
Z2	113	±3.72	0.72	1489	2033	±56	1.9	14
Z1	100	±2.59	2.80	1418	1910	±56	3.5	14
Z3	97	±2.96	3.19	1488	2086	±60	1.1	14
Z7	94	±2.52	3.75	1519	1865	±41	4.7	12
Z6	67	±2.58	7.95	1187	1520	±41	9.9	12
KA1	128	±4.04		466	2724	±52		11
KA3	119	±3.86	1.58	508	2509	±54	2.9	13
KZ3	110	±4.05	3.09	1686	2197	±21	9.4	13
KZ1	107	±2.69	4.30	1651	2056	±40	10.2	12

* Protein and total calories include those derived from yeast.

$$\text{PE (Probable Error)} = \frac{\sigma \times 0.6745}{\sqrt{n}}$$

$$\sigma \text{ (Standard Deviation)} = \sqrt{\frac{\sum d^2}{n-1}}$$

$\sum d^2$ = sum of the deviations from the mean, squared.

n = number of observations

$$\text{SR (Significance Ratio)} = \frac{D}{PEd}$$

When the value is 3 or over, the difference between the 2 means is considered significant.

D = difference between two means.

$$\text{PEd (Probable Error of the Difference)} = \sqrt{(\text{PE mean 1})^2 + (\text{PE mean 2})^2}$$

TABLE IV

A COMPARISON OF THE MEAN GAINS IN WEIGHT AND FOOD INTAKE OF GROUPS OF NEPHRECTOMIZED AND INTACT RATS OVER A PERIOD OF 56 DAYS

Group	Mean gain in weight	Significance ratio	Total food eaten*	
			Calories	Significance ratio
	gms.			
A1	128		2308	
KA1	128	0.00	2724	5.7
A3	129		2414	
KA3	119	2.05	2509	1.4
Z1	100		1910	
KZ1	107	1.88	2056	2.1
Z3	97		2086	
KZ3	110	2.59	2197	6.7
P1	128		2091	
KA1	128	0.00	2724	11.4
KZ1	107	4.81	2056	0.7
P3	133		2338	
KA3	119	2.35	2509	2.8
KZ3	110	3.78	2197	4.0
Z1	100		1910	
KA1	128	5.83	2724	10.7
Z3	97		2086	
KA3	119	4.52	2509	5.2

* Total calories include those derived from yeast.

controls (A3) (Table V). Yet a comparison of Z3 with KZ3 (Table IV), shows no significant difference between their gains in weight. Rats with two kidneys seem, therefore, to tolerate such an excessive level of dietary protein no better than rats with only one kidney. Possibly the shorter experimental period which we have used may account for the differences of these results from those of Smith and Jones (6), who found a retarded rate of growth in nephrectomized rats if they were confined to a ration containing more than a moderate amount of the protein constituent.

Within the limits of our experiment, it appears that the growth of

nephrectomized rats is not hindered by a 50 per cent level of casein in the diet (Table IV). The mean gain in weight of the P1 group equals that of the KA1 group and exceeds that of the KZ1 group. The same statement applies to the P3 group when it is compared with intact groups of rats fed the control or the high protein rations, each supplemented with the same yeast allowance as P3 received.

TABLE V

COMPARISON OF THE GAINS IN BODY WEIGHT AND OF FOOD CONSUMED BY RATS WHOSE RATIONS CONTAINED DIFFERENT LEVELS OF PROTEIN

Group	Mean gain in weight	Significance ratio	Food calories ingested*	
			Total	Significance ratio
	gms.			
A4	129		2678	
Z4	118	1.44	2175	5.2
A5	112		2358	
Z5	114	0.42	2238	2.0
A2	143		2483	
Z2	113	5.83	2033	6.2
P2	135	1.64	2222	5.4
A1	128		2308	
Z1	100	8.02	1910	5.3
P1	128	0.00	2091	3.9
KA1	128		2724	
KZ1	107	4.33	2056	10.2
A3	129		2414	
Z3	97	7.64	2086	4.5
P3	133	0.74	2338	1.5
KA3	119		2509	
KZ3	110	1.61	2197	5.4
A7	108		2293	
Z7	94	4.13	1865	8.6
A6	83		1888	
Z6	67	3.39	1520	5.2

* Total calories include those derived from yeast.

A revived interest in growth has been stimulated by some recent papers (9, 12). It is timely, therefore, to make a comparison of the ability of rats to grow when they are limited to dietaries differing either in the quantity of casein, or in the amount or combinations of the vitamin B (B_1), G (B_2), or B complex supplements.

The mean gains in weight of all groups appear in Table III, while in Table V each experimental group is compared with its respective A or KA control. Among all the 21 groups represented, the most rapid growth was achieved by the A2 group of rats. A comparison of this group with the P groups and also with the Z group which gained the most weight, shows that over 66 per cent of the rats fed a ration containing 50 per cent of casein (Table VI) grew as well as those eating a diet in which the casein

TABLE VI

COMPARISON OF THE GAINS IN WEIGHT OF THE P GROUPS, ALSO OF THE MOST RAPID-GROWING Z GROUP, WITH THE A GROUP OF RATS WHICH GAINED THE MOST IN BODY WEIGHT

Group	Mean gain in weight	Significance ratio
	gms.	
A2	143	
P2	135	1.62
P3	133	1.74
P1	128	3.04
Z4	118	3.64

constituent was 18 per cent. Our results are thus in agreement with those of Sherman and Gloy (3) and with Smith and Moïse (10) in experiments in which rations containing 60 per cent of casein were fed to young rats. The most rapidly growing Z group of rats, however, made gains slightly inferior to those of the animals in the A2 group. This comparison is irrespective of differences in allowances of vitamin B, G, and B complex supplements.

The groups Z1, Z2, and Z3, which were given a ration identical in vitamin supplements with those consumed by the three P groups, made mean gains in weight inferior to those of the animals in the P groups (Table VII) and also to those of their own controls (Table V) A1, A2, and A3 respectively. Of the remaining Z groups whose vitamin adjuvants differed from those given to the rats in the P groups, Z4 and Z5 have grown as well as their

controls, but Z6 and Z7 made gains in weight which are significantly poorer than their own controls.

Thus, though the ration containing 50 per cent of casein permits an animal to grow as well as its controls whether they have two kidneys (Table IV) or only one (Table V), a ration having 90 per cent of this protein produces growth equivalent to that of controls in only 28 per cent of the nephrectomized groups (2 out of 7 cases, Table V) and in 50 per cent of those whose rats had two kidneys (1 out of 2 cases, Table V). The best growth reported by Osborne and Mendel (11) was attained by the use of a ration containing 35 per cent of casein, and the most rapid rate of

TABLE VII

COMPARISON OF THE GAINS IN WEIGHT OF P AND Z GROUPS OF RATS WHICH CONSUMED THE SAME AMOUNTS OF YEAST SUPPLEMENTS

Group	Mean gain in weight	Significance ratio
	gms.	
P1	128	
Z1	100	6.52
P2	135	
Z2	113	4.32
P3	133	
Z3	97	5.66

growth yet reported was secured by Anderson and Smith (12), who fed to their rats a stock diet which furnished 33 per cent of protein from one source and 35 per cent from another food mixture, both of which were freely available to the rats at all times.

From the evidence available at present, the optimum level of dietary protein for rats lies between 18 and 50 per cent and probably between 30 and 40. Our results and those quoted above do not confirm Slonaker's findings.

The supplements which promoted the best growth in rats restricted to the control diet are 0.8 gm. of yeast, or 1.6 gm. of yeast fortified by tikitiki extract (Table III). Those which stimulated growth best when the rats were confined to the high-protein ration, are tikitiki extract or autoclaved yeast when either one was added to the highest level of yeast (1.6 gm.). In comparing the Z groups among themselves (Table III), 0.8 gm. of yeast

is just as effective as 1.6 gm. supplemented with either autoclaved yeast or tikitiki extract, but when comparing the mean gain in weight of Z2 with its control, A2 (Table V), 0.8 gm. of yeast is less effective than the other two supplements, for Z4 and Z5 are the only ones among all the seven Z groups which made as satisfactory gains as their respective controls.

The need of rats maintained on high-protein regimens for additional B or G vitamins, besides that furnished by yeast, has been emphasized by Hassan and Drummond (1). On the other hand, a comparison of the gains in weight of groups of rats which received *yeast alone* as the source of the B vitamins (Table VIII) does not support the thesis of Reader and Drummond (2), for increasing the supply of yeast to 1.6 gm. did not improve the rate of growth of any of the groups which we have studied.

As yeast is still the chief source of the B vitamins used in biological in-

TABLE VIII

GAINS IN BODY WEIGHT AND FOOD INTAKE OF GROUPS OF RATS GIVEN DIETS CONTAINING 18, 50, AND 90 PER CENT OF CASEIN SUPPLEMENTED BY DIFFERENT LEVELS OF YEAST

Group	Mean gain in weight	Significance ratio	Food calories ingested*	
			Total	Significance ratio
	gms.			
A1	128		2308	
A2	143	3.53	2483	2.5
A3	129	0.26	2414	1.6
P1	128		2091	
P2	135	1.42	2222	5.7
P3	133	0.88	2338	7.2
Z1	100		1910	
Z2	113	2.87	2033	1.6
Z3	97	0.76	2086	2.1
KA1	128		2724	
KA3	119	1.58	2509	2.9
KZ1	107		2056	
KZ3	110	0.12	2197	3.1
Z2	113		1903	
Z3	97	2.93	1959	0.7

* Total calories include those derived from yeast.

vestigations, a comparison is given in Table VIII of the growth-stimulating effects of the three levels used as supplements to each of the three rations. The groups which made the best gains in weight are A2, P2, and Z2. However, in the case of only the A2 group is there a significant difference in the weight gained over that attained by the other groups fed on the same level of protein (A1 and A3), but whose yeast allowance was different. It appears that the 0.8 gm. level of yeast is the best growth-stimulant of any of the quantities of yeast alone which we have used.

When, in the absence of yeast, either the control or the high protein ration was supplemented with a large supply of vitamin G from autoclaved yeast and a minimum of B from tikitiki extract (groups A6 and Z6, Table III), the growth-response of the rats was meagre. Likewise, if an excessive amount of B together with a very small allowance of G were furnished, the mean gains in weight were still poor. Results similar to these have been observed by Graham and Griffith (13). It is likely that in each case the vitamin which was given in smaller amount was inadequate in quantity, or that some other of the B vitamins necessary for growth either is not furnished at all, or is supplied in insufficient quantity under the conditions of this study.

There are many conflicting reports concerning the ability of rats to grow on high-protein rations in a normal manner or as well as their controls fed the ordinary levels of protein. In view of our results it seems possible that the lack of agreement may be due to a deficiency of one or more of the B vitamins.

It is quite possible that under paired feeding conditions our results would be quite different from those obtained when rats have free access to an abundant food supply. One of us (L. D. F.) is at present investigating this phase of the subject.[4] Data from a very limited number of rats at present point to a better utilization of the protein-rich food for the growth of rats, than of the ration containing 18 per cent of casein.

In a comparison of the caloric intake of our groups of experimental rats with that of their respective controls (Table V), the significance ratio figures in all but two cases indicate that the experimental animals consumed a significantly less amount of food. This comparison holds even with the A4 and Z4 groups, whose mean gains in weight are not significantly different. This condition suggests, as indicated above, that the two high-protein rations used, in terms of calories, are more efficient in the production of growth in young rats than is a diet containing 18 per cent of protein. A

[4] In the Physiology Laboratory of Wellesley College.

smaller energy intake on the part of rats confined to a protein-rich dietary, has also been observed by Smith and Moïse (10).

Summary

Unilateral nephrectomy *per se* did not impair the ability of rats to grow when fed a ration containing any one of the three given levels of casein.

Rats restricted to a diet containing 50 per cent of casein grew as well as their controls having 18 per cent of this protein in their rations and better than rats confined to a 90 per cent casein-containing dietary.

Only 28 per cent of the groups maintained on the food containing 90 per cent of casein made gains in weight equal to those of their controls which had 18 per cent of casein in their ration.

Of the five groups of rats fed the highest level of protein supplemented with *yeast alone*, 80 per cent of the groups made inferior gains in weight in comparison with their controls and all were inferior to the groups of rats whose diet contained 50 per cent of casein.

The optimum level of dietary protein for the production of rapid growth, appears to lie between 18 and 50 per cent.

The best growth-promoting supplement, when yeast was used *alone*, was 0.8 gm. This quantity fed with the control diet produced significantly better growth than either the 0.4 or the 1.6 gm. level. With the rations containing more protein, however, any one of the levels of yeast served equally well for stimulating growth.

The best growth attained on the ration containing 18 per cent of casein was by the use of 0.8 gm. of yeast and also by the 1.6 gm. level of yeast plus tikitiki extract.

The best growth recorded for the highest protein diet occurred when the 1.6 gm. level of yeast was fortified with either tikitiki extract or with autoclaved yeast.

In the absence of yeast, both an excess of B with a minimum of G, or an excess of G along with a small amount of B are unsatisfactory growth stimulants. Probably autoclaved yeast and tikitiki extract furnish only some of the necessary B vitamins.

There is some evidence that better utilization of food for growth is possible by feeding high protein rations in contrast to the control diet containing 18 per cent of casein.

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CALCIUM AND PHOSPHORUS IN THE DEVELOPMENT OF THE TURKEY EMBRYO*

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PROGRESS in the determination of causes of embryo mortality seems to be dependent in a measure upon accurate knowledge of the metabolism of the developing embryo. A review of the literature of this subject is given by Needham (1).

Experimental Method

The Bronze turkey eggs used in this experiment were produced at the Western Kentucky Experiment Substation at Princeton, Kentucky. They were collected for a period of thirteen days, shipped to Lexington, Kentucky, and placed in an electric (Petersime) incubator. The average temperature was 99.75°F. throughout the period of incubation with a relative humidity of 64 per cent. All eggs were turned four times daily. At twenty-four hour intervals, six eggs weighing 80 to 90 grams each were removed and stored in a refrigeration room at 28 to 30°F. This effectively stopped embryonic development and preserved the eggs until analyzed at a later date. The embryos were removed from the eggs, compared with previously standardized specimens, freed of all adhering yolk, including that within the body cavity, dried on filter paper, transferred to silica dishes, weighed, and dried to a constant weight in an electric oven at 100C.° The dry embryos were burned to a white ash, which was dissolved in hydrochloric acid, made to a definite volume with distilled water, and calcium and phosphorus determined in separate aliquots. Calcium was determined by the McCrudden volumetric method (2) and phosphorus by the volumetric method of the Association of Official Agricultural Chemists (3).

Results and Discussion

The present study of the Bronze turkey embryo is concerned with: 1.—embryonic growth as measured by wet weight, dry weight, and ash content; 2.—percentage of moisture; 3.—growth cycles; and 4.—calcium

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

and phosphorus content. Comparisons of these studies will be made with those reported on the chick embryo.

The data covering the growth of the turkey embryo as measured by wet weight, dry weight, per cent moisture, and ash content, are given in Table I and Figure 1. The daily increase in wet weight, dry weight, and ash con-

TABLE I

GROWTH, MOISTURE AND ASH CONTENT OF THE TURKEY EMBRYO

Day	Average weight of eggs	Number of embryos	Wet weight grams	Dry weight grams	Per cent moisture	Ash content grams
5	84.2	5	.0156	.0050	67.95	
6	84.5	2	.0330	.0104	68.48	
7	83.5	4	.0797	.0204	74.40	.0004
8	83.7	3	.1903	.0279	85.34	.0013
9	84.5	2	.5121	.0586	88.56	.0044
10	86.3	3	.5823	.0576	90.11	.0043
11	82.5	2	.9492	.0719	92.43	.0085
12	82.5	4	1.7670	.1583	91.04	.0138
13	83.0	5	3.9664	.2719	93.15	.0269
14	82.7	6	4.4310	.3715	91.62	.0319
15	83.7	3	5.6975	.6530	88.54	.0523
16	85.6	5	6.2538	.9141	85.38	.0669
17	84.8	6	7.0468	1.2354	82.47	.0865
18	81.0	2	12.1482	2.2035	81.86	.1501
19	83.7	3	15.4144	3.1686	79.44	.1923
20	84.5	4	16.5000	3.2900	80.09	.2175
21	83.5	4	23.8900	4.8200	79.81	.3250
22	84.0	4	27.5100	5.8200	78.86	.3950
23	85.8	5	33.2100	6.8600	79.35	.4900
24	83.4	5	36.9300	7.8900	78.63	.5840
25	85.0	5	44.3600	9.2900	79.05	.6900
26	84.2	5	49.3600	11.3700	76.96	.7920

tent is very small until the twelfth day after which the increments of growth noticeably increase. As shown in Figures 1 and 2, embryonic growth is divided into three distinct phases or cycles with definite periods of retardation between the ninth and tenth days and between the nineteenth and twentieth days. These periods of retardation appear at approximately the same relative time as those appearing on the ninth and sixteenth days for the chick embryo. Needham (*loc. cit.*) has shown that during the early period of incubation up to and including the seventh day, the chick embryo uses carbohydrate as the source of energy. This period corresponds to the first nine days of the turkey embryo's life and it will

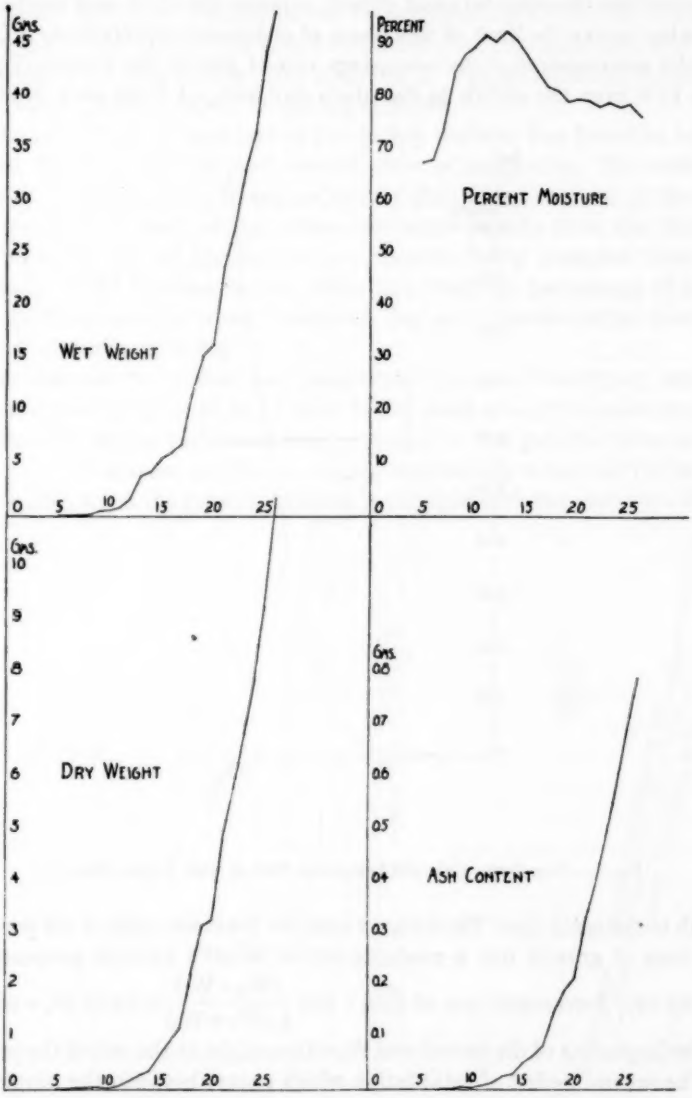

FIG. 1.—The Growth of the Bronze Turkey Embryo as Measured by: Wet Weight, Ash Content and Per Cent of Moisture.

be noted that the retardation of growth between the ninth and tenth days probably marks the limit of this phase of embryonic metabolism. It is especially noticeable that the percentage rate of gain in dry matter (Figure 2) is 71.0 from the eighth to the ninth day and −1.7 per cent from the

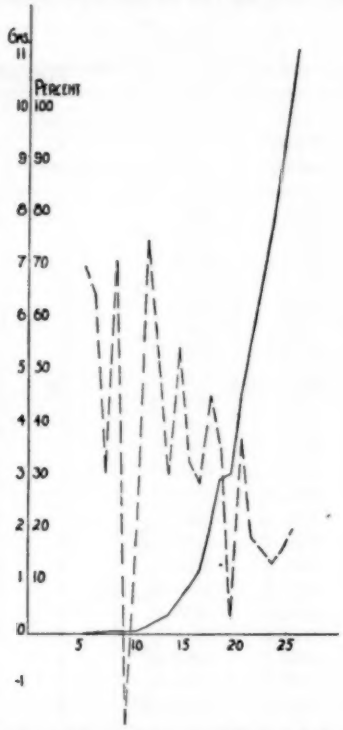

FIG. 2.—Cumulative Gain and Percentage Rate of Gain Turkey Embryo.

ninth to the tenth day. The formula used for the calculation of the percentage rate of growth was a modification of Minot's formula proposed by Brody (4). Percentage rate of gain $= 100 \frac{(W_2 - W_1)}{\frac{1}{2}(W_1 + W_2)}$ in which W_1 = weight at the beginning of the period and W_2 = the weight at the end of the period.

The second period of retardation which occurs between the nineteenth and twentieth days gives a definite decrease in percentage rate of gain, and a decided pause in dry weight, wet weight, and ash content. Needham has discussed the utilization of protein during the period from the seventh

to the sixteenth day of incubation and the metabolization of fat thereafter. The break in the curve of the turkey embryo corresponds to the change in materials metabolized. This break in the curve of the chick coincides with the peak of embryonic mortality as shown by Payne (5). Mortality curves have not as yet been given for turkey embryos.

The percentage of moisture in the turkey embryo was found to be the lowest between the fifth and seventh days of incubation. The moisture content at this period was approximately that of the content of the egg. The moisture content of the embryo increases rapidly from the sixth to the eleventh day of incubation and remains fairly constant from the eleventh to the fourteenth day. After this time the percentage of moisture declines steadily to the nineteenth day and remains rather constant until the twenty-fifth day.

The data on the calcium and phosphorus content of the turkey embryo are presented in Table II and Figure 3. The total amount of calcium gives a somewhat similar picture to that presented by the growth curves of the embryo. The increase in calcium content is relatively slow until the seventeenth day, when the growth becomes more rapid with the exception of the

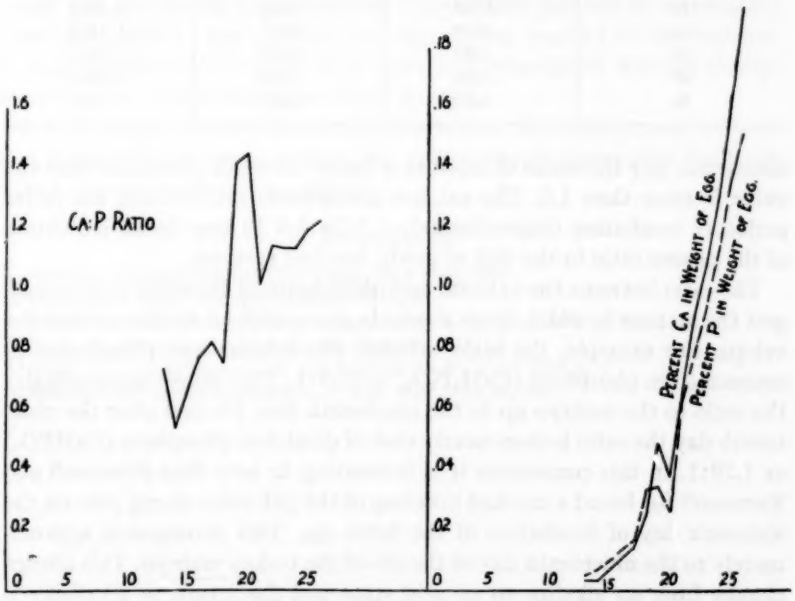

FIG. 3.—Calcium and Phosphorus in the Developing Turkey Embryo.

decrease from the nineteenth to the twentieth day. The rapid increase in calcium content is resumed after the twentieth day. The phosphorus curve follows very closely that of calcium, with the same decrease from the nineteenth to twentieth day. The calcium-phosphorus ratio with phosphorus remaining constant at 1.0 is shown in Figure 3 and Table II. Until the

TABLE II

CALCIUM AND PHOSPHORUS CONTENT OF TURKEY EMBRYOS

Day	Calcium (Ca) grams	Phosphorus (P) grams	Ca:P ratio
13	.0031	.0042	.74:1
14	.0031	.0057	.54:1
15	.0057	.0085	.67:1
16	.0092	.0118	.78:1
17	.0119	.0143	.83:1
18	.0207	.0274	.76:1
19	.0409	.0289	1.42:1
20	.0321	.0222	1.45:1
21	.0543	.0534	1.02:1
22	.0764	.0664	1.15:1
23	.0928	.0812	1.14:1
24	.1081	.0948	1.14:1
25	.1388	.1160	1.20:1
26	.1632	.1327	1.23:1

nineteenth day the value of calcium is below 1.0 while after that time the value is more than 1.0. The calcium-phosphorus ratio during the latter period of incubation (approximately 1.2 Ca:1.0 P) may be an indication of the proper ratio in the diet of newly hatched turkeys.

The ratio between the calcium and phosphorus in the embryo may suggest the manner in which these elements are combined as the embryo develops. For example, the ratio between the calcium and phosphorus in monocalcium phosphate ($CaH_4P_2O_8$) is 0.65:1. This closely approximates the ratio in the embryo up to the nineteenth day. On and after the nineteenth day the ratio is more nearly that of dicalcium phosphate ($CaHPO_4$) or 1.29:1. In this connection it is interesting to note that Romanoff and Romanoff (6) found a marked lowering of the pH value of egg yolk on the sixteenth day of incubation of the hen's egg. This corresponds approximately to the nineteenth day of the life of the turkey embryo. This abrupt change from an alkaline to an acid state and the return to alkalinity is explained by them as follows, "The above-mentioned sudden drop in pH

value under both natural and artificial incubation is possibly related to the natural depression of growth in the life span of the embryo." It is possible, however, that this change of pH is ascribable to a sudden change in the form of the calcium-phosphorus compound present, as discussed above.

Summary

1. A study was made of 1.—embryonic growth as measured by wet weight, dry weight and ash content; 2.—per cent of moisture; 3.—growth cycles; 4.—calcium and phosphorus content of the Bronze turkey embryo.

2. The daily increase in wet weight, dry weight, and ash content was very small until the twelfth day, after which time increments of growth noticeably increase.

3. The increase in calcium and phosphorus content was relatively slow until the seventeenth day.

4. The calcium-phosphorus ratio was less than 1.0 until the nineteenth day of incubation, after which time it exceeded 1.0.

5. Turkey embryo growth is divided into three distinct phases or cycles, with definite periods of retardation between the ninth and tenth and between the nineteenth and twentieth days. These periods of retardation were also noted in the calcium and phosphorus content of the embryo. Certain correlations between these periods of retardation and the change in the type of food metabolized are suggested.

6. It is suggested that the calcium content of the embryo may be used as a measure of growth provided the yolk material drawn into the body cavity is removed before analysis.

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THE INFLUENCE OF THE PRECEDING DIET UPON THE RATE OF GLUCOSE ABSORPTION AND GLYCOGEN SYNTHESIS

By

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CARBOHYDRATE tolerance, as measured by the height of the blood sugar curve following the ingestion of a given quantity of glucose, is varied by the nature of the preceding diet (1-8). The tolerance is lower after fasting than when food has been taken, reduced by a high protein or a high fat diet, and increased by a diet high in carbohydrate. The influence of the preceding diet upon the glucose tolerance may be either through an effect upon the rate of glucose absorption from the intestine, the rate of hepatic and muscle glycogen synthesis, the rate of glucose absorption by the tissues (with storage, oxidation or conversion to fat), or all of these factors. The present experiments were designed to determine the influence of the preceding diet upon the first two of these factors in the albino rat.

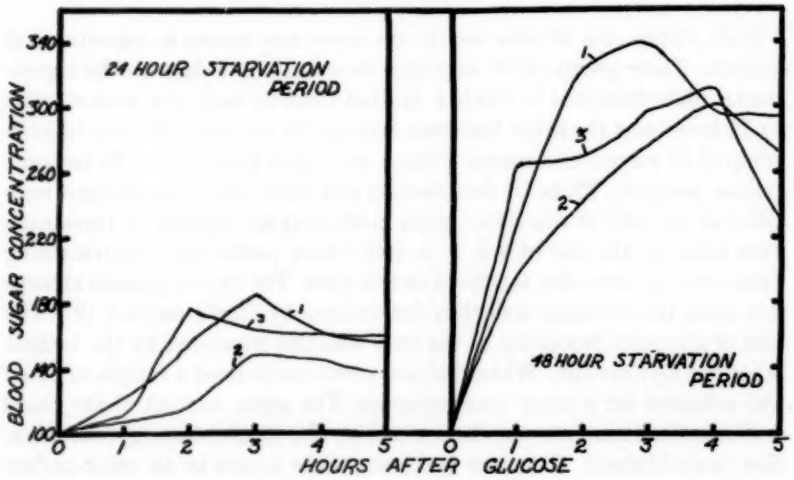
METHODS

Male albino rats of very nearly the same age served as experimental subjects. Three groups of 42 rats each were fed for 45 days on the experimental diets described in Table I. Half of these in each case were starved for 24 hours and the other half were starved for 48 hours. Six rats of each group of 21 served as controls. Fifteen were each given 1.0 cc. 50 per cent glucose solution. Three of the controls and three receiving glucose were killed at the end of one hour, being given amytal. Groups of three each were killed at the end of a 2, 3, 4, and 5 hour period and the remaining three controls were also sacrificed at this time. The rate of glucose absorption from the intestine was thus determined by Cori's method (9). The rate of glycogen deposition in the liver was also measured by the technic described by Cori (10). When the rats were anesthetized a sample of blood was collected for a sugar determination. The sugar content of the blood and intestinal contents was determined by the Shaffer-Somogyi modification (unpublished). The liver specimens were frozen in an ether-carbon dioxide snow mixture and weighed while frozen. Skeletal muscle specimens

TABLE I

Ingredients	Diet 1	Diet 2	Diet 3
Commercial casein	65	15	15
Raw cornstarch	0	50	0
Powdered yeast (Fleischmann's)	10	10	10
Salt mixture (Osborne and Mendel)	4	4	4
Lard	15	15	37
Cod liver oil	6	6	6
Cellulose (powdered)	0	0	28
Composition			
Protein	65	17	17
Carbohydrate	22	22	44
Fat	4	50	4
Proportion of Calories			
Protein	56	14	14
Carbohydrate	2	43	3
Fat	42	43	83

were rapidly removed from the lower limbs and treated in the same manner. The glycogen samples were received into boiling KOH solution and glycogen determined by Sahyun's method (11).



FIGS. 1 AND 2.

TABLE II

TABLE II

Length of absorp. period hr.	24 hours starvation							48 hours starvation						
	Body surface sq. cm.	Liver weight gm.	Liver glycogen per cent	Muscle glycogen per cent	Glucose fed gm.	Glucose in G. I. tract gm.	Blood sugar conc. mg. per cent	Body surface sq. cm.	Liver weight gm.	Liver glycogen per cent	Muscle glycogen per cent	Glucose fed gm.	Glucose in G. I. tract gm.	Blood sugar conc. mg. per cent
Diet 1—Protein														
0	485	8.4	0.20	0.18	0.00	0.00	93	391	6.3	0.45	0.22	0.00	0.00	68
1	549	9.5	0.12	0.19	2.75	1.65	112	396	5.8	0.88	0.21	1.98	1.31	171
2	544	9.1	1.41	0.21	2.62	1.38	154	417	7.1	1.46	0.27	2.08	1.53	236
3	527	9.2	2.40	0.25	2.64	1.35	185	398	6.1	2.17	0.33	1.99	0.80	247
4	533	9.7	2.28	0.30	2.96	1.39	164	401	6.8	2.84	0.26	2.01	0.63	222
5	572	10.0	2.27	0.32	2.76	0.71	163	388	7.1	3.33	0.42	2.01	0.43	215
0	577	8.5	0.15	0.19	0.00	0.00	105	373	5.4	0.46	0.18	0.00	0.00	78
Diet 2—Carbohydrate														
0	543	8.9	1.05	0.21	0.00	0.00	124	417	6.6	0.18	0.21	0.00	0.00	53
1	534	10.4	0.97	0.18	2.67	1.55		403	6.3	0.62	0.25	2.02	1.05	101
2	515	10.3	1.99	0.26	2.58	1.43	141	423	6.5	0.88	0.24	2.03	1.16	119
3	434	8.3	2.09	0.31	2.34	1.22	180	427	7.2	2.58	0.27	2.01	1.13	138
4	508	9.6	2.89	0.33	2.67	0.55	157	428	6.3	2.64	0.22	2.14	0.81	158
5	499	8.8	3.14	0.38	2.67	0.55	157	428	6.6	3.28	0.30	2.15	0.47	135
0	486	8.4	0.71	0.21	0.00	0.00	113	369	5.2	0.09	0.23	0.00	0.00	47
Diet 3—Fat														
0	547	10.0	0.88	0.29	0.00	0.00	106	425	6.0	0.29	0.17	0.00	0.00	73
1	510	10.3	1.08	0.28	2.56	1.62	117	423	6.1	0.55	0.17	2.11	1.61	177
2	541	12.7	1.25	0.34	2.70	1.47	188	422	6.5	1.58	0.31	2.11	1.19	179
3	514	10.2	2.13	0.40	2.57	1.19	172	415	6.9	2.21	0.40	2.07	0.80	195
4	548	11.3	2.66	0.42	2.73	1.51	170	396	6.4	2.48	0.17	1.95	0.81	199
5	498	9.9	3.05	0.52	2.49	0.79	166	449	7.8	3.23	0.44	2.25	0.52	152
0	531	9.1	0.39	0.24	0.00	0.00	107	398	6.2	0.13	0.23	0.00	0.00	59

TABLE III

Length of absorp. period hr.	24 hours starvation						48 hours starvation					
	Percentage sugar absorbed	Absorption coefficient gm.	Liver wt. per sq. dcm. body surface gm.	Glycogen formed per 100 gm. liver gm.	Liver glycogen formed per 100 sq. cm. body surface gm.	Percentage of sugar absorbed deposited as liver glycogen	Percentage sugar absorbed	Absorption coefficient gm.	Liver wt. per sq. dcm. body surface gm.	Glycogen formed per 100 gm. liver gm.	Liver glycogen formed per 100 sq. cm. body surface gm.	Percentage of sugar absorbed deposited as liver glycogen
Diet 1—Protein												
1	40.0	0.201	1.73	0.00	0.000	1.0	33.8	0.169	1.46	0.43	0.006	3.5
2	47.4	0.114	1.67	1.21	0.024	10.5	26.4	0.066	1.70	1 01	0.017	12.9
3	48.8	0.082	1.74	2.20	0.042	17.1	59.9	0.099	1.53	1.72	0.026	8.8
4	53.1	0.074	1.82	2.08	0.041	13.9	68.7	0.086	1.70	2.39	0.041	11.9
5	74.3	0.071	1.75	2.07	0.040	11.3	78.6	0.081	1.83	2.88	0.053	13.1
Diet 2—Carbohydrate												
1	42.0	0.210	1.95	0.00	0.000	1.0	48.0	(0.241)	1.56	0.44	0.007	2.9
2	44.6	0.113	2.00	0.94	0.022	9.7	42.9	0.103	1.54	0.72	0.011	5.3
3	47.8	0.086	1.91	1.29	0.023	8.9	43.8	0.068	1.69	2.44	0.041	20.1
4	70.4	0.088	1.89	2.18	0.038	10.8	62.2	0.078	1.47	2.52	0.037	11.8
5	79.4	0.085	1.76	2.43	0.040	9.4	78.0	0.078	1.54	3.18	0.049	12.5
Diet 3—Fat												
1	36.8	0.184	2.02	0.20	0.004	2.2	23.7	0.118	1.44	0.26	0.004	3.4
2	46.0	0.115	2.34	0.45	0.015	6.5	43.7	0.109	1.54	1.32	0.020	9.2
3	53.3	0.089	1.98	1.53	0.030	11.2	61.4	0.103	1.66	1.96	0.032	10.3
4	44.6	0.056	2.06	2.16	0.044	19.8	58.6	0.072	1.62	2.27	0.037	12.8
5	69.1	0.068	1.98	2.65	0.053	15.6	77.0	0.077	1.73	3.10	0.054	14.0

Results

The summarized figures presented in Tables II and III are all averages for three rats. The blood sugar curves (Figs. 1 and 2) after glucose following the various diets show relations which might be expected (4).

Cori (9) used as his absorption coefficient the amount of sugar absorbed per hour in relation to body weight. We have found (12) that the absorption rate bears a more constant relation to body surface than body weight. Consequently the rate of glucose absorption per 100 sq. cm. body surface per hour has been used as the absorption coefficient. Cori (9) found the absorption coefficient constant from hour to hour, a straight line relationship. We find a marked falling off in the absorption rate during successive hours after giving glucose, a finding which will be discussed in more detail elsewhere.

The glucose absorption coefficient (Table IV) is highest after the carbohydrate diet and lowest after the high fat diet. It is obvious that changes in the absorption rate can hardly contribute to any variation in the glucose tolerance curve, but on the contrary make it necessary for some other factor to over-compensate the changes in the absorption rate. Our figures confirm Cori's (13) observation that the absorption coefficient for glucose tends to be lower after a 48-hour starvation period than after 24 hours.

Table IV

Average Absorption Coefficients (Amount of Sugar (gm.) Absorbed per 100 Square Centimeters Body Surface per Hour)

	All absorp. periods		First single hour absorption period		All absorp. periods except first single hour	
Hours after food	24	48	24	48	24	48
Diet 1 Protein	0.100	0.100	0.201	0.169	0.080	0.084
Diet 2 Carbohydrate	0.116	0.114	0.210	(0.241)	0.093	0.082
Diet 3 Fat	0.102	0.096	0.184	0.118	0.082	0.090

The average fasting glycogen content of skeletal muscle (Table V) after the various diets shows no notable change with the possible exception of the high figure for the six fat fed rats after 24 to 29 hours of starvation. The figures for this group were uniformly high.

The fasting liver glycogen in Table V was naturally highest in the high carbohydrate group after 24 hours starvation. The fat-fed rats had more glycogen in their liver than the high-protein-fed ones, just the reverse of

TABLE V
FASTING GLYCOGEN CONTENT

	Skeletal muscle per cent			Liver per cent		
Hours without food	24–29	48–53	Av.	24–29	48–53	Av.
Diet 1 Protein	0.18	0.20	0.19	0.17	0.45	0.31
Diet 2 Carbohydrate	0.21	0.22	0.21	0.88	0.13	0.51
Diet 3 Fat	0.26	0.20	0.23	0.63	0.21	0.42

findings reported (14) on rats receiving very similar diets without any starvation. On further starvation both the fat and carbohydrate fed rats lost glycogen while the protein fed rat liver apparently gained it. This may have been partly the result of the loss of water (14).

Hepatic glycogen was synthesized (Table III) just about as rapidly on all three diets after 24 hours starvation. The rate was lower if anything in the high carbohydrate group, just the reverse of what we had expected. This may be due to the higher fasting (24 hours) liver glycogen content of these livers for after the administration of glucose after a 48 hour fasting period (Table III) glycogen synthesis was most rapid on the high carbohydrate diet group. The concentration of glycogen, though, was practically the same in all three groups at the end of the five hour absorption period.

The changes in the glycogen content of skeletal muscles were not of such a degree that they might have any significant influence upon the blood sugar tolerance curves.

CONCLUSIONS

We have found no influence of the preceding diet upon either the rate of glucose absorption from the intestine or the rate of glycogen synthesis in either the liver or skeletal muscle of such a nature that it might account in any degree for the variations in the blood glucose tolerance curves produced by diet. Consequently we must conclude that these variations are produced almost if not entirely by changes in the rate of removal of glucose by the tissues. Our experiments do not show definitely the mechanism which leads to this variable rate of glucose uptake by the tissues which may be due to changes in storage of the sugar as such, as glycogen, or in the rate of oxidation. We know that storage as glycogen in the tissues (skeletal muscle) is unimportant and it seems unlikely that there could be such continued differences in the rate of glucose storage as such. Changes in the rate of glucose oxidation seem by far the most likely explanation.

The current view that the influence of the preceding diet (8) upon the blood sugar tolerance curve is due to the level at which the sugar metabolizing hormone has been produced and hence is available (e.g., a high carbohydrate diet would call for more insulin than fasting and after it a lower glucose tolerance curve would result) fits in well with this picture. Insulin is known to be without influence upon the rate of hepatic glycogen deposition in the normal animal (15) and there is no evidence that it may affect the rate of absorption of glucose from the intestine.

Summary

In the albino rat the preceding diet, as varied by being composed chiefly of protein, carbohydrate, or fat, was without significant effect, after 24 and 48 hours of starvation, upon the rate of absorption of administered glucose from the intestine or the rate of glycogen deposition in the liver or skeletal muscle. It is therefore concluded that the variations in the blood glucose tolerance curve produced by the preceding diet are due in large part, if not entirely, to changes in the rate of glucose uptake by tissues other than the liver probably produced by variations in the rate of glucose oxidation.

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THE POSSIBILITY OF GLUCONEOGENESIS FROM FAT

II. THE EFFECT OF HIGH FAT DIETS ON THE RESPIRATORY METABOLISM AND KETOSIS OF MAN*

By

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INTRODUCTION

SHOULD fatty acids in considerable quantity be transformed to carbohydrate and the latter remain unoxidized for some time in the mammalian body, the effect inevitably would be a depression of the respiratory quotient below the level of ordinary fasting. Quotients above unity indicating the reverse reaction are easily obtained, but the rarity of very low quotients, say below 0.67, reliably reported, indicates that it certainly is difficult to induce this transformation. Obviously it was to the advantage of primitive man as well as to the beasts in a state of nature that fat should be formed more easily than resolved again. Nature is a better banker than man. Spending from savings and the conversion of credits (fat) to small coin (sugar) should be difficult, if not impossible.

Ordinarily the laying on of fat takes place very gradually and the R.Q. need not exceed unity. Perhaps the reverse process also takes place very little at a time, short periods of conversion alternating with short periods of combustion, or, possibly, the two running concurrently, so that the details are obscured. It is evident that if combustion kept up with conversion there would be no lowering of the R.Q. This situation has long been recognized, but so far as we can discover, nobody has made any attempt avowedly to devise conditions wherein the two component reactions might conceivably be separated in time sufficiently for independent recognition. One of us has shown (1) recently that this is possible both temporally and spatially with a fatty seed (castor bean) where sugar is formed from fat in the endosperm and oxidized (in part) in the young plant.

In a student problem in nutrition one of the authors (J) appeared to have an unusual tolerance for fat. This observation coincidentally was

* A large part of the data in this paper was contained in a thesis submitted by Estelle E. Hawley to the University of Rochester in partial fulfillment of the requirements for the Ph.D. degree.

made, as Dr. I. McQuarrie was carrying out in this laboratory analyses of respired air from epileptic children on ketogenic diets, in which some quite low respiratory quotients were observed. It occurred to us that by prolonging a tolerable, high fat diet for several days it might be possible to induce a condition of glycogen hunger, with no other adequate means of supply except a sudden, great excess of soluble fatty acids, and that in this combination of circumstances the tendency would be first toward conversion of fat to sugar (low respiratory quotients) and sometime later toward combustion (high quotients). Maintenance of partial undernutrition would serve to deplete the glycogen stores. The tissues, liver in particular, would lay first claim to any sugar formed and would gradually pay out its gain to the muscles and other organs after the first full flood of fat absorption had passed. This was the hypothesis. Easily digestible and assimilable fat which contained a wide distribution of fatty acids would be the one of choice. Just what level of fat ingestion and what fatty acid-to-glucose ratio in the food would be most favorable to the purpose remained to be seen. The occurrence of such a sequence of quotients would not of itself prove the conversion of fat to carbohydrate, unless there were no other plausible explanation.

Procedure

The first experiment occurred in January and February, 1930, with two subjects, H and J, who were at the same time the operators, each for the other, of the respiration apparatus (Benedict Universal). They took for 6 days a diet containing 74 per cent of the calories as fat (F.A.: G by weight = 1.5). This was followed immediately by a diet of the same general character containing 84 per cent fat (F.A.: G by weight = 3.0) for 7 days and this, in turn, by a third with 94 per cent fat (F.A.: G = 4.1) for 5 days. The last diet consisted exclusively of 4X cream (actually 37.5 per cent fat by analysis).

A second experiment was carried out by these two subjects in April and May, 1930. The intermediate diet was maintained as before. A third subject (M) participated on the 4.1:1 ratio diet only.

The third experiment occurred in October and November when the high-ratio diet was taken again by subjects M and J, and in February, 1931, it was taken by a fourth subject (V.S.) who, while on the diet submitted twice to a shivering experiment, which proved especially interesting from the standpoint of the respiratory quotient. In November, 1931, a student (W) volunteered to take the high-fat diet and was studied by one of us using the Tissot-Haldane procedure (Chart 4). Finally in November, 1932,

another student (F) took the all-cream diet and was studied, under supervision, by another student competent in air analysis procedure (Chart 4).

Aside from the respiratory quotient it was, of course, necessary for purposes of interpretation to have knowledge of the acidosis, if any, produced and any indication of conversion of fat to carbohydrate which might be obtainable from analysis of the blood. A rise of blood sugar, should it occur, ought not to be missed. Accordingly in three subjects total and ammonia nitrogens and acetone bodies in two fractions were determined in the urine and acetone bodies, CO_2-combining power (occasionally), total fat, and blood sugar, all in whole blood. Both urine and blood were collected at three-hour intervals, the collection in both cases coming immediately, or soon after the termination of a respiratory determination. Most complete data were obtained for the second and third experiments on the 4.1:1 ratio diet and consequently the discussion will concern this diet chiefly.

The daily routine for the subject who had been on diet for several days, typically was as follows on the experiment days:

1. Bladder emptied on arriving at laboratory.
2. One-half to one hour rest on bed.
3. Basal R.Q.'s immediately after which basal bloods were taken.
4. Basal urine collected.
5. High-fat test meal eaten (usually before 10.00 a.m.).
6. R.Q.'s at 1½ hour intervals from time of finishing meal.
7. Blood and urines collected at 3-hour intervals.

The majority of the experiments ran to 6 or 7½ hours, a few to 9 and two to 10½ and 11 hours.

Methods of analysis.—Total N and ammonia N in the urine were determined by the macro Kjeldahl and Folin aeration, respectively, urine and blood acetones by the Behre-Benedict (2) distillations and Hubbard's (3) titration methods. Blood sugar by Folin-Wu, blood fat by Bloor's (4) oxidation method and CO_2-combining power by the Van Slyke and Neill's (5) manometric method.[1] The respiratory quotients were determined in the majority of the daily runs by the Benedict "Universal" method only. The apparatus was provided with a graphic spirometer for better control of the exact time for throwing of the valve and the oxygen was measured into the apparatus after saturation with moisture by a carefully calibrated wet-test meter. In several experiments the R.Q.'s were checked by parallel

[1] We are indebted to Dr. E. S. Nasset for these determinations.

determinations by the Tissot-Haldane procedure,[2] employing the du-Vigneaud (6) modification of the Haldane. The agreement was not always perfect, but in the majority of cases was within the range of variation for successive determination by the same method (see below). The former apparatus was checked frequently by burning alcohol in the circuit and the latter by outside air analyses. No respiration experiment with the Benedict apparatus has been included for which there was not a graphic record of the respirations, showing that the valve was thrown correctly, *and a satisfactory alcohol check*. No experiment by the Tissot method is included, for which there was not a satisfactory outside-air analysis, checking the accuracy of the technic. These check data are omitted merely for conservation of space. A further check of importance to be noted is that most of the experiments on subjects H and J were carried out alternately by the same apparatus. A leaky apparatus could not give a low R.Q. on one subject and a high one on the other.

Altogether there were 32 experiment "days," meaning that respiratory quotients were taken for at least six hours following a test meal. On 18 "days" urine and blood analyses were made with more or less completeness.

Results

Space does not permit the presentation of more than a third of all of the tabular results of this study. For two subjects the following tables (I to IV) show the effects of a high and a low F.A. to G ratio at two different dates for each. The second low-ratio diet was taken about 11 weeks after the first and the second high ratio diet about 15 weeks after the first. For another subject three successive days are shown in Table V. These will illustrate the important factors concerned. Supporting data in addition will be found in the charts.

The low-ration diet was the same for subjects H and J. It was prepared in the diet kitchen of the department and consisted of three meals, except on experimental days, when only two were taken. Breakfast contained eggs, bread, butter, sugar, 4X cream and coffee; lunch, the same with the addition of peanut butter and orange juice; dinner, eggs, meat, milk chocolate, sugar, 4X cream, and coffee. The composition from standard tables was: protein 115, fat 286, carbohydrate 109 gms. The total fatty acid was calculated at 310.5 gms. and total glucose at 206.4, making the F.A.: G ratio 1.5. The total calories were too high.

[2] We are indebted to Dr. R. W. Swift for most of these measurements.

TABLE I

SUBJECT H—RESPIRATORY METABOLISM AND KETOSIS

F.A.:G ratio = 1.5:1; 6th day of diet; meal contained 45 gm. F.A.

Date 1930	Time of period from meal	R.Q.	Liters per hr.		Heat per hr.		Per minute		Urine, mgms./hr.			Blood values, mgm. /100 cc.		CO_2 comb. power vol. %
			O_2	CO_2	Total	Cal./sq. m.	Pulse	Resp.	Total N	NH_3N	Total† acetone	Sugar	Total acetone†	
Jan. 19	Basal	0.76	11.24	8.54	53.4	35.1	76	14	356	15	10.8	92	42	—
	1½ hrs.	0.72	12.95	8.29	61.5	40.0	80	16	—	—	—	—	—	—
	3 hrs.	0.73	11.76	8.64	55.5	36.5	76	16	—	—	—	99	—	—
	4½ hrs.	0.74	11.84	8.80	56.0	36.8	80	16	—	—	—	—	—	—
	6 hrs.	0.73	11.34	8.23	53.4	35.1	76	14	—	—	—	91	39.5	—
	7½ hrs.	0.73	11.22	8.19	51.7	34.8	76	14	—	—	—	—	—	—
Apr. 8	Basal	0.69	11.58	7.97	54.3	35.7	66	9	329	41	17.9	82	14.4	53.2
	1½ hrs.	0.70	13.33	9.38	62.5	41.1	68	8	—	—	—	—	—	—
	3 hrs.	0.51*	13.65	6.96*	64.0	42.1	69	9	288	42	17.9	94	8.6	46.2
	4½ hrs.	0.70	13.04	9.16	61.1	41.1	74	8	—	—	—	—	—	—
	6 hrs.	0.68	12.65	8.60	59.3	39.0	69	8	436	38	19.8	95	13.6	51.6
	7½ hrs.	0.67	12.14	8.17	56.9	37.4	66	10	—	—	—	—	—	—

* Probable error in weighing CO_2—Heat computed for R.Q. of 0.707.

† "Total acetone" denotes the sum of preformed acetone, diacetic acid and β-hydroxybutyric acid expressed as acetone.

TABLE II

SUBJECT J—RESPIRATORY METABOLISM AND KETOSIS

F.A.:G ratio = 1.5:1; 6th day of diet; meal contained 90 gm. F.A.

Date 1930	Time of period from meal	R.Q.	Liters per hr.		Heat per hr.		Per minute		Urine, mgms./hr.			Blood values, mgm. /100 cc.		CO_2 comb. power vol. %
			O_2	CO_2	Total	Cal./sq. m.	Pulse	Resp.	Total N	NH_3N	Total acetone*	Sugar	Total acetone†	
Jan. 19	Basal	0.74	15.63	11.50	73.8	36.9	58	16	383	35		88	5.4	—
	1 hr.	0.66	18.58	12.19	87.1	45.8	60	16	—	—	—	—	—	—
	2½ hrs.	0.66	19.80	13.13	92.8	48.9	60	18	—	—	—	98	—	—
	4 hrs.	—	**	10.42	—	—	58	18	—	—	—	—	—	—
	5½ hrs.	0.67	16.59	11.12	77.7	40.9	60	14	—	—	—	91	5.9	—
	7 hrs.	0.73	15.63	11.42	73.7	38.8	63	16	—	—	—	—	—	—
Apr. 8	Basal	—	—	—	—	—	—	—	598	31	—	91	8.9	54.4
	1 hr.	0.75	20.85	15.61	98.8	52.0	62	18	—	—	—	—	—	—
	2½ hrs.	0.74	22.57	16.65	106.6	56.1	76	16	532	39	—	100	7.1	51.6
	4 hrs.	0.72	18.74	13.56	88.2	46.4	60	18	—	—	—	—	—	—
	5½ hrs.	0.72	17.12	12.40	80.6	42.4	56	20	600	41	—	107	9.2	51.8
	7 hrs.	0.74	16.93	12.60	80.1	42.2	70	18	—	—	—	—	—	—

* Negative to ferric chloride. ** Oxygen reading lost.

† "Total acetone" denotes the sum of preformed acetone, diacetic acid and β-hydroxybutyric acid, expressed as acetone.

TABLE III

SUBJECT H—RESPIRATORY METABOLISM AND KETOSIS

High fat; F.A.:G ratio 4.1:1 5th day of diet; meal contained 111 gm. F.A.

Date 1930	Time of period from meal	R.Q.	Liters per hr.		Cal. per hr.		Per minute		In urine, mgm. per hr.			In blood, mgm. per 100 cc.		
			O_2	CO_2	Total	Sq. m.	Pue	Resp.	Total N	NH_3N	Total acetone	Sugar	Total acetone	Fat
Feb. 2	Basal	—	—	—	—	—	—	—	98	33	369	65	48	—
	1½ hr.	0.60	13.22	8.12	61.9	40.7	84	8	—	—	—	—	—	—
	3 hrs.	—*	—	—	—	—	—	—	—	—	—	—	—	—
	4½ hrs.	0.62	12.62	7.88	59.2	38.9	80	12	—	—	—	72	43	—
	6 hrs.	0.72	12.77	9.01	59.9	39.4	88	10	—	—	—	—	—	—
	7½ hrs.	0.61	13.33	8.18	62.4	41.1	88	12	163	49	350	—	40.8	—
May 25	Basal	0.64	11.81	7.61	55.3	36.4	72	8	197	71	196	67	35	498
	1½ hrs.	—†	—	—	—	—	—	—	—	—	—	—	—	—
	3 hrs.	0.66	13.09	8.65	61.3	40.3	66	9	214	81	216	65	36	515
	4½ hrs.	0.67	13.32	8.92	62.4	41.1	70	9	—	—	—	—	—	—
	6 hrs.	0.68	13.69	9.27	64.2	42.2	72	11	216	80	206	74	28	‡

* Period omitted because of discomfort of subject.

† Period omitted to run alcohol check.

‡ Lost.

TABLE IV

SUBJECT J—RESPIRATORY METABOLISM AND KETOSIS

High fat; F.A.:G ratio 4.1:1; 5th day on diet; meal contained 222 gm. F.A.

Date 1930	Time of period from meal	R.Q.	Liters per hr.		Cal. per hr.		Per minute		In urine, mgm. per hr.			In blood, mgm. per 100 cc.		
			O_2	CO_2	Total	Sq. m.	Pulse	Resp.	Total N	NH_3N	Total acetone	Sugar	Total acetone	Fat
Feb. 2	Basal	—	—	—	—	—	—	—	259	8	31.7	84	22.5	—
	1 hr.	0.75	21.41	13.10	101.5	53.5	76	16	—	—	—	—	—	—
	2½ hrs.	0.72	24.57	15.09	115.5	60.7	63	16	405	29	45.0	80	22.9	—
	4 hrs.	0.67	20.30	13.59	95.1	57.7	66	18	—	—	—	—	—	—
	5½ hrs.	—*	—	—	—	—	—	—	222	18	47.0	72	28.1	—
	7 hrs.	0.71	18.80	13.41	88.4	46.5	70	21	—	—	—	—	—	—
	9 hrs.	—	—	—	—	—	—	—	175	27	63.0	—	26.9	—
May 25	Basal	0.71	15.29	10.86	71.6	37.7	54	16	253	35	71.5	71	12.0	697
	1 hr.	—†	—	—	—	—	—	—	—	—	—	—	—	—
	2½ hrs.	0.74	20.46	15.06	99.6	50.9	62	19	307	38	80.7	71	39.0	837
	4 hrs.	0.72	19.37	14.03	92.2	48.0	66	20	—	—	—	—	—	—
	5½ hrs.	0.74	17.41	12.85	82.3	43.0	60	22	290	26	75.5	73	34.2	798

* Subject fell asleep, ruining period.

† Period omitted to run alcohol check (see 1½ hr. on H., Table III).

In Table I may be seen contrasting effects, partly of the diet as a whole and partly of the single test meal, taken on the sixth day of the diet in each case, but taken in the second instance after the subject was well accustomed to the diet. The meal contained 45 gm. calculated fatty acid. The data for urine and blood are not so complete on the January experimental day as on the April day. To speak of the respiratory metabolism first, it is evident that the subject was more restless in the earlier experiment. The pulse and respiration indicate this, and possibly the R.Q.'s also. The principal cause was alimentary discomfort. There is evidence in the data for heat production that absorption was by no means so complete in the first experiment as in the second. The specific dynamic action was highest the first period (1½ hours) following ingestion of the meal in the former, but was maximal at the second period (3 hours) in the latter, and had not yet returned to basal at 7½ hours. The principal reason for the lower R.Q.'s in the second trial with this diet therefore would seem to be associated with the better utilization. The term applies to events beyond the alimentary wall as well as to those within; for while the figures for the urinary acetone bodies ("total acetone" throughout means all three acetone products combined and expressed as acetone) are higher in the later experiment, those for the blood total acetone are much lower both before and after the meal. Placing the greater emphasis on blood acetone, this subject illustrates the principle of adaptation in the sense of an acquired tolerance for a high-fat diet which has been noted by Wigglesworth (7) for the rat. It is seen also in Tables III and VI.

It is the more remarkable that the respiratory signs of altered metabolism (low R.Q.'s) should be so much more pronounced in the second trial. The CO_2-combining power of the whole blood was lowest when the specific dynamic action was highest. There was no driving off of CO_2. (It happened as a matter of ill-luck that the figure for CO_2 at the third hour was questionable, at the very time when, according to convention, it should have been high—the combining power at this point being lowest.) The ammonia figure for the urine indicates no change from basal at the 3-hr. collection; but it must be borne in mind that the urine represents the collective metabolism for the past three hours while the blood analyses indicate only a momentary condition.

There is a greater rise from basal in the blood sugar this (second) experimental day, but the meal contained a considerable amount of carbohydrate and therefore it probably is meaningless as evidence of gluconeogenesis. The same comment applies to the next experiment.

Subject J on the same general diet and following a test meal with twice the fatty acid value of that for subject H, on corresponding days of the diet, manifested a different reaction, at least as regards the R.Q.'s. (Table II.) They run just as uniformly high in the second trial as they did for subject H in the first; also they run even lower in the first trial than they did for subject H in the second. The specific dynamic action is in agreement with that of subject H in showing more rapid absorption the second, than the first trial, while the ketosis, so far as the record goes, is so slightly changed that very little can be said about it. Surely it does not help the interpretation of the R.Q.'s. Nor does the CO_2-combining power. The fall of a few volumes per cent following a fatty meal is something seen quite regularly in these experiments but it is seldom reflected in the respiration record in any way. Perhaps alveolar R.Q.'s after the manner of those recently reported by Carpenter and Lee (8) would be more significant, in a situation like this. There is nothing of interest in the other blood or urinary findings, except that the higher basal total urinary N the second trial may indicate some hang-over effect from the evening meal of the day before.

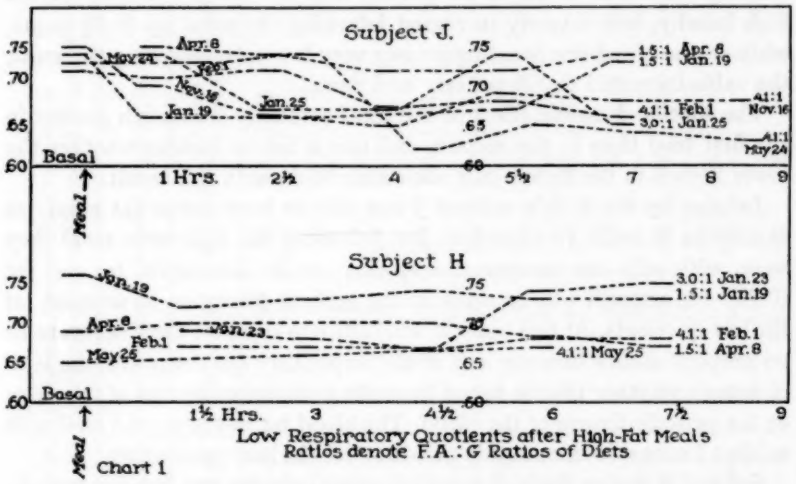
It should be emphasized again that the two experiments on H and J were run on the same day each time, alternating period by period on the same machine and since the operation of the apparatus also was identical for the two subjects, the differing results on the two cannot be accounted for by different conditions of the apparatus. Subject J also seemed to tolerate the diet and the test meal subjectively somewhat better the second time than the first. This is borne out by the continued low figures for ketosis. With subject H (a woman) the improved tolerance was reflected in the ketosis but not in the R.Q.'s with subject J decidedly more in the R.Q.'s. Since the test meal contained twice as much fatty acid equivalent for subject J, and there was at no time any significant production of ketone bodies, it is clear that the male subject had the higher tolerance for fat. This is in agreement with a recent report by Deuel and Gulick (9).

Immediately following the first low-ratio diet both subjects H and J took an intermediate diet having a F.A.:G ratio of 3:1 for 7 days and then the really high-ratio diet for five days. There were two experimental days, the 4th and 6th for each subject on the 3:1 diet, but for want of space they are omitted from the tables. Certain significant results from these days, however, are contained in Chart 1.

The same sort of comparisons for the first time and the second time on the high-ratio diet are seen in Tables III and IV. The test meal consisted exclusively of 4X cream. Again the days of the diet on which the metabo-

lism experiment was performed corresponded for the two subjects, and the test meal of subject J was twice that of subject H in fatty acid equivalent.

The R.Q.'s for corresponding periods of the two trials were not so different as they were on the low-ratio diet. The respiratory data are regrettably incomplete. The diet was exceedingly hard to take for some subjects and the somnolence and lassitude consequent upon the ketosis are subversive of the utmost willingness in the world to coöperate. Furthermore, the two



Low Respiratory Quotients after High-Fat Meals
Ratios denote F.A. : G Ratios of Diets

Chart 1

subjects were alternately active and quiet in serving as observer and subject (see page 526) which probably accounts for some irregularities. With subject H (Table III) the ketosis was again much greater than with subject J (Table IV), notwithstanding the much smaller meal of cream. The high basal figures for urinary and blood acetone indicate that the allowance of fat was much greater than the former subject could properly metabolize. Nevertheless she stuck to the regimen and in the second trial again achieved a measure of adaptation, as shown by the lower ketosis. There was less ammonia available for neutralization of the ketosis in the first trial than in the second, possibly because of somewhat better absorption of the proteins of the cream in the latter. The specific dynamic action, perhaps also as a consequence of the better absorption, was higher in the absolute sense the second trial. The exact basal heat production not being known the first trial, the percentage increase expressing the specific dynamic action cannot be given. In both instances absorption seems to have

been quite slow, for the heat production at the end of the day was greater than at the third or second periods. On the low-ratio meal (Tables I and II) the highest effect came at the first or second period, for both subjects.

Subject J (Table IV) seems to have handled the high-ratio diet and test meal better the first trial than the second. The basal urinary ketosis was low initially the first time, increased up to the fourth period only 50 per cent, and finally at nine hours to 100 per cent, while the blood ketosis scarcely increased at all. In the second trial the urinary ketosis started high basally, and scarcely increased following the meal up to 5½ hours, while in the blood the basal figure was very low and, following the meal, the value increased to 300 per cent and more.

The specific dynamic effect of the meal evidently was much greater in the first trial than in the second, and one is led to wonder whether the lower ketosis in the former had something to do with this result.

Judging by the R.Q.'s, subject J was able to burn butter fat nearly as rapidly as it could be absorbed; for, following the high-ratio meal they were, with only one exception, very close to the theoretical for this fat (0.72). An attempt will be made in the general discussion to account for the low quotients. At this point it will suffice to say that there seems to be no proportionality between any of the respiratory quotients and the level of ketosis whether this be taken from the cumulative figures of the urine or the periodic figures of the blood. The blood fat in the second trial with subject J runs a course roughly parallel with the heat production.

Subject M was on the high-ratio (all-cream) diet for two five-day periods, one in May and the other in October. During the first period three successive respiratory studies were made on the 3rd, 4th, and 5th days of the diet (Table V). On the 4th and 5th days the ketosis was traced by urinary analysis rather completely, and on the 4th and 5th days quite completely also by blood analysis. Only one respiration period was lost (May 21, 2nd period following the meal).

On the first experimental day the lowest R.Q.'s (0.68) were found at the 1st and 3rd periods (1½ and 4½ hours following the test meal). The same is true of the second day (0.69 and 0.70) although in this instance the intermediate period showed a higher R.Q. (0.75). The third day none lower than 0.72, occurring at the 3rd and 6th hour, was recorded. On each of the three days the R.Q. dipped below the basal level soon after the meal and then rose progressively to 0.77 or 0.78 at the end of the day (see Chart 2). Note that with the exception of a single determination (3d hr.) on the second day the general levels follow the order of the days, i.e., first, lowest,

TABLE V

SUBJECT M—RESPIRATORY METABOLISM AND ACETONE-BODY PRODUCTION

High-fat diet; F.A.:G=4.1.:1; 3rd, 4th, and 5th days of diet; meal contained 227 gms. F.A.

Date 1930	Time of period from meal	R.Q.	Liters per hr.		Cal. per hr.		Per minute		In urine, mgms. per hr.			In blood, mgm. per 100 cc.			CO_2 comb. power vol. %
			O_2	CO_2	Total	Sq. m.	Pulse	Resp.	Total N	NH_3N	Total acetone	Sugar	Total acetone	Fat	
May 21	Basal	0.74	15.59	11.59	73.9	33.8	66	13	—	—	$FeCl_3$ +	—	—	—	—
	1½ hrs.	0.68	20.17	13.75	94.5	43.2	69	12	—	—	—	—	—	—	—
	3 hrs.	omitted	—	—	—	—	—	—	—	—	+	—	—	—	—
	4½ hrs.	0.68	18.84	12.80	88.3	40.3	69	14	—	—	—	—	—	—	—
	6 hrs.	0.72	18.50	13.24	86.9	39.7	80	14	—	—	+	—	—	—	—
	7½ hrs.	0.78	17.14	13.30	71.8	37.4	78	12	—	—	—	—	—	—	—
May 22	Basal	0.71	17.03	12.08	75.0	35.1	60	12	285	48	15.2	97	27	—	—
	1½ hrs.	0.69	19.34	13.21	90.8	41.4	60	13	—	—	—	—	—	—	—
	3 hrs.	0.75	19.71	14.74	92.2	42.2	78	12	447	30	18.0	—	—	—	—
	4½ hrs.	0.70	19.02	13.29	89.1	40.7	72	14	—	—	—	—	—	—	—
	6 hrs.	0.73	19.21	14.08	90.5	41.3	75	14	384	33	60.0	81	18	—	—
	7½ hrs.	0.77	17.77	13.49	84.7	38.7	66	14	—	—	—	—	—	—	—
	9 hrs.	0.77	17.03	13.03	81.0	37.0	75	10	36	53	41.6	—	—	—	—
May 23	Basal	0.74	17.57	13.03	83.1	37.9	90	10	285	49	15.2	91	15	372	43
	1½ hrs.	0.73	20.45	14.97	96.4	44.0	78	10	—	—	—	85	61.3	735	—
	3 hrs.	0.72	20.83	14.92	97.9	44.7	90	10	372	36	36.0	94	24.0	815	39
	4½ hrs.	0.73	20.05	14.65	94.5	43.2	87	12	—	—	—	80	34.0	764	—
	6 hrs.	0.72	20.02	14.42	94.1	43.0	86	12	333	61	62.0	70	28.0	728	—
	7½ hrs.	0.74	18.04	14.00	85.2	38.9	87	10	—	—	—	70	24.0	737	—
	9 hrs.	0.76	18.83	14.25	89.4	40.8	86	14	—	—	—	72	48.0	790	—
	10½ hrs.	0.78	18.34	13.22	85.9	39.2	86	14	250	50	60	—	—	—	—

second intermediate and third, highest. This particular feature will be commented upon in the general discussion. The averages for the 3 days were .716, .730 and .740.

The specific dynamic effect of the meal was greatest on the second and third experimental days at the end of three hours following the meal. This coincides with the highest blood fat on the one day when it was followed, and it is probable that the same would have been shown in the period which was lost on the first day. This agrees fairly well with subject J, whose highest specific dynamic action occurred at the second respiration period (2½ and 2¾ hours) following his all-cream test meal. The heat curve fell fairly regularly from this high point to the end of the day. (Compare with the record of specific dynamic action of dogs fed emulsified fat by Murlin and Lusk, 10.) Even at 10½ hours (May 23), however, it was still above the basal level for the day.

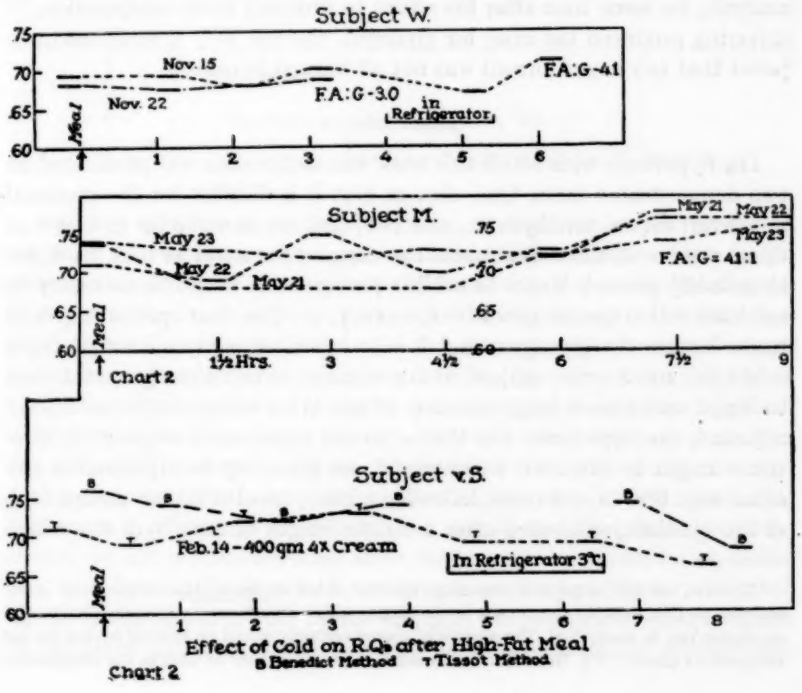
In the progressively higher basals on the 2nd and 3rd experimental days there is just a little evidence for the old idea of luxus consumption. But it might equally well be called simply a survival of the specific dynamic effect of the previous day's diet as a whole. It would be interesting to know how long it might have remained high.

The highest urinary production of acetone bodies occurred at the sixth hour (second three-hour collection) following the test meal in both the 2nd and 3rd experimental days, showing simply that the greatest *elimination* came between the third and sixth hour. If the greatest *production* in the tissues can be told from the blood figures for "total acetone," that process must have been most active (on the 3rd day at least) in the first hour-and-a-half period. It was not accompanied by a low R.Q. Had there occurred such a coincidence, we might have had reason to regret that bloods were not taken at this time on the 1st and 2nd days when the R.Q.'s were lowest. In short, we again see in these results no near relationship between low quotients and ketosis. Possibly the respiration periods were quite too short for such a purpose. Further discussion of the evidence will follow.

Adaptation to high fat.—Except for the more normal quotients on the second, and still more on the third experimental day, there is in this experiment with subject M no evidence of adaptation in so short a time. Perhaps, however, normal quotients constitute as significant a sign as any, and the term should be broadened to include better oxidation (Chart 2).

With subject H also there was *in successive days* on the same diet (not shown in tables) no evidence of adaptation in the ketosis figures; rather

the contrary. Such adaptation as is noticeable, when similar experimental days are laid side by side, occurred many weeks after the original observation. A time element seems to be necessary. The only subject who manifested any sign of adaptation or improved tolerance, so far as the ketosis was concerned, within a few days was subject J, and in his case the change was no greater than the reverse sign, i.e., loss of tolerance within the same time, for subject H. Both, therefore, are probably only day to day variations. It was not possible, because of the rigorous character of the diet, to prolong the observations at a given level sufficiently to determine the minimal time within which the human subject would acquire a definitely greater tolerance. What we are certain of is that the cha..ge was apparent in subject H three months after the first trial, i.e., from January to April, on the low-ratio diet and four months (February to May) on the high-ratio diet. Meantime a considerable change of season had occurred, and, as Cori and Cori (11) have shown for the rat, season plays an important part in determining the completeness of fat metabolism. In the fasting rat, however, ketonuria was much accentuated in the summer months



while in the present instance both ketonuria and ketosis were diminished in the spring as compared with the winter. The explanation does not appear to lie in a change of external factors, but of internal.

Effect of low environmental temperature.—On the hypothesis that the low respiratory quotients soon after a meal of high fat were due to the need for glycogen, any other means of producing the same need at a time when much fatty acid is still available, should induce the same response. Shivering is known to be a good means of removing glycogen. Two of the subjects therefore (V.S. and W., chart 2) were placed in the refrigerator which had been used by Dr. Swift (11a, 12) for his experiments on "The Effects of Low Environmental Temperature upon Metabolism" to see whether shivering would cause a lowering of the R.Q. The refrigerator period came at a time (4–5 hours after the test meal) when the R.Q. usually is rising. The effect is quite pronounced with both subjects. Subject V.S., who was a very thin person, shivered violently for 80 minutes of his sojourn. Subject W, who was quite stout, shivered very little. Accordingly, V.S. showed a greater effect on the R.Q. and this persisted, as shown by the direct air analysis, for some time after his return to ordinary room temperature. If shivering produced the need for glycogen, the low R.Q.'s would seem to prove that any sugar formed was not all burned at once.[3]

Discussion

The hypothesis with which this work was undertaken was predicated on two demonstrated facts. One, that at best it is difficult for the mammal to convert fat to carbohydrate, and two, that the demand for glycogen as tissue reserve at times dominates the demand for sugar as fuel. Both are abundantly proved. Hence to exhibit conversion it would be necessary to establish rather special conditions; namely, 1.—just that special degree of tissue hunger for glycogen, and 2.—no other adequate source of sugar available, and 3.—the subject, at the moment of test already accustomed to digest and absorb large amounts of fat. With these conditions rightly adjusted, the hypothesis was that a certain sequence of respiratory quotients might be obtained which could not plausibly be explained in any other way. First would come, following a heavy meal of fat, an abrupt drop of the quotient, succeeded after a certain length of time by a rise which

[3] There is, however, a question regarding the effect of low environmental temperature on dissociation of CO_2 from its compounds in the tissues which should receive attention before this conclusion can be made final. The superficial tissues certainly would be affected by the low air temperature (about 3°C). Note that the Benedict method could not be used in the refrigerator.

would go at least as high as the original basals. Averaged for the day the quotients should stand near the expected level for combustion of the ingested fat. Gregg (13) in this laboratory calculated the theoretical quotient for butter fat to be 0.72, instead of the usually accepted 0.707 for mixed food or body fats, the higher level being explained by the presence of lower fatty acids in butter.

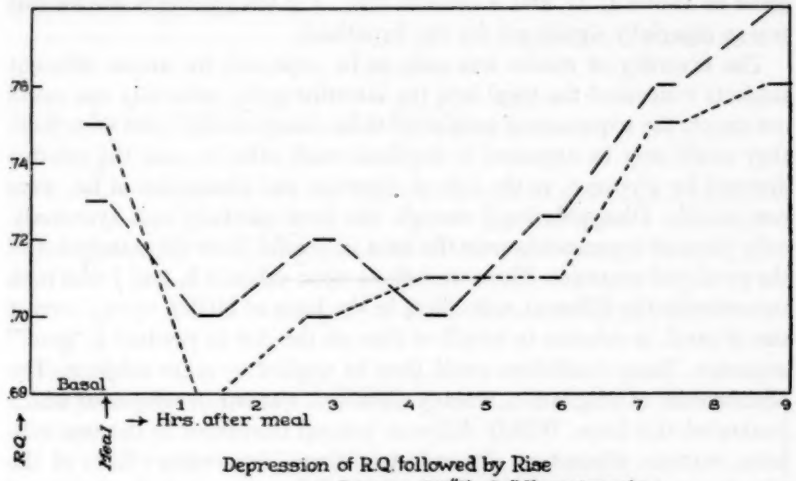


Chart 3

The first question to be discussed is, to what extent was the predicted sequence of respiratory quotients realized? Chart 3 shows that as the average result of seven "early" experiments there was a marked depression below the basal level, and that this was followed by a recovery to a level higher than basals. The same chart shows that the average of three successive days with subject M gave a smaller depression but a still higher recovery following. Chart 1 contains several sequences for subject J which exhibit a fall from basal with no, or at most only a transitory, recovery and for subject H, several which ran continuously low throughout the day. Charts 2 and 4 contain some of the most satisfactory (to the hypothesis) sequences in individual experiments, i.e., quotients falling far below theoretical for fat combustion soon after the test meal and recovering above this level late in the day. Such a sequence cannot possibly be accounted for by the secretion of HCl by the stomach, producing a relative alkalosis in the blood and a subsequent reversal as absorption of the chlorine ions

occurs; for in the first place a fat meat produces relatively little gastric juice, second, neutralization in the alimentary tract where very little protein is present to bind up the acid, must take place very promptly after evacuation into the intestine, and, in the third place, the CO_2-combining power of the blood always indicates acidosis, if any change, at the point where the quotients are lowest (several other determinations than those given in Tables I, II, and V confirm this). The low quotients are for this reason especially significant for the hypothesis.

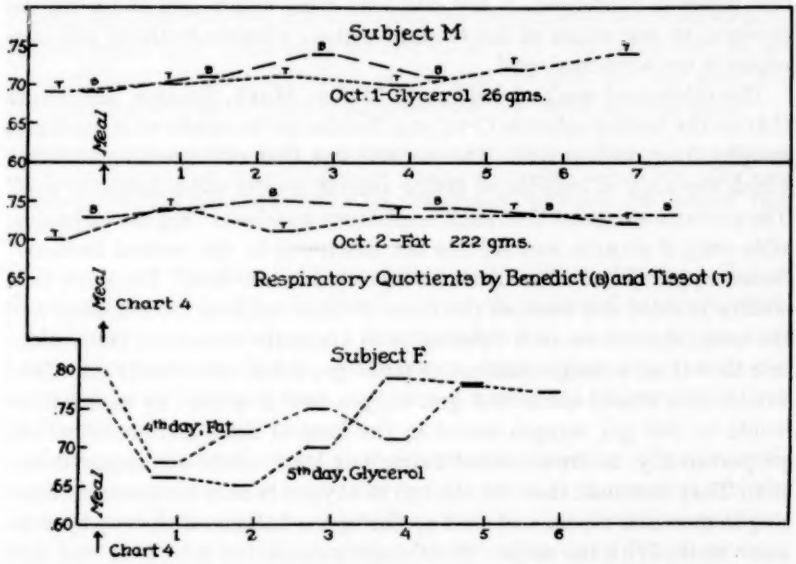
The diversity of results was only to be expected; for unless different subjects evacuated the meal into the intestine quite uniformly one could not expect the sequences of quotients[4] to be closely similar; and even then, they could only be expected to duplicate each other in case the relative demand for glycogen, to the rate of digestion and absorption of fat, were comparable. Disappointingly enough, the most carefully and systematically planned experiments were the least successful from the standpoint of the predicted sequence. These were those upon subjects H and J who took successively the different ratio diets in the hope of hitting upon a correct size of meal, in relation to length of time on the diet to produce a "good" sequence. These conditions could then be applied to other subjects. The phenomenon of adaptation, already discussed, was one development which frustrated this hope. Widely different natural tolerances in the two subjects, extreme alimentary discomfort at times, depressing effects of the ketosis causing occasional loss of periods, alternating periods of rest and great activity, and, finally, probably too much food (calories), were others.

The three subjects, M, V.S., and F, who gave the most "satisfactory" sequences each had been on the all-cream diet only a short time. V.S. had fasted a full day and then had taken nothing but 4X cream for three days. Subject M showed a larger depression of the quotient on the third day of the diet, than on the fourth and fifth. Indeed, as noted on page 534 the general level of the quotients followed the order of the days themselves, indicating acquired capacity to oxidize the fat as such, without any visible improvement from the standpoint of ketosis. This statement sounds contradictory, but will be cleared up presently in discussion of the significance of low quotients in special relation to ketosis.

Concluding the point under discussion at present, it should be stated that while there is a certain gratification in having demonstrated that the

[4] Many of the irregularities in the R.Q.'s shown in Tables I to IV are believed to be due to irregular evacuations of the fat from the stomach into the intestine, the subjects being alternatively quiet and active.

predicted sequence can be obtained, the authors do not consider that low quotients occurring in other than this particular sequence are without significance. Indeed, if we could know the exact order of evacuation from the stomach, the glycogen status of the liver and the food mixtures of the portal vein from period to period, a fortuitously occurring low quotient might turn out to be even more significant than an orderly succession of them followed by high ones.



The next question to be discussed, naturally, is, can these quotients be explained in any other way than by conversion of fatty acid to carbohydrate?

The opinion in this country has come to prevail, mainly through the teaching of Professor Lusk and his pupils, of whom the present senior author has been a convinced believer in this doctrine, that quotients below 0.69 are not trustworthy either in normal or diabetic subjects and can only be accounted for in the diabetic by extreme ketosis or by maximum conversion of protein to sugar. Everybody will admit that quotients below 0.69 are very exceptional and appear only under unusual circumstances of metabolism. Until this work was begun the senior author had never seen such quotients in adult human subjects except when there was good reason to believe that the technic was faulty. From personal participation

in the experiments here reported, and others to be reported later, he is now convinced that they are not only possible but in some subjects they occur quite regularly on high-fat diets.

Looking into the literature with the present hypothesis in mind, the conviction has grown that many such quotients, hitherto regarded as unreliable, probably are reliable. They have been obtained by reliable methods and frequently under conditions not greatly dissimilar to those with which this paper is concerned. A few citations must suffice and those will be confined to researches of the modern period, wherein methods still currently in use were employed.

The celebrated work of Lehmann, Müller, Munk, Senator, and Zuntz (14) on the fasting subjects Cetti and Breithaupt is replete with quotients ranging from 0.65 to 0.68. The method was that of Geppert and Zuntz which certainly is capable of giving correct results when properly used. The authors recognize that such respiratory quotients "appear to be possible only if protein and fat are not destroyed in the normal fashion." Some oxygen rich residue "must be deposited or excreted." The latter possibility is ruled out because the feces of these subjects were normal and the urine showed no such substances in adequate quantity. They calculate that if an average quotient of 0.687 prevailed for a whole day Cetti would have stored some 30.4 gm. oxygen and in a ten-day period there would be 300 gm. oxygen stored in the form of some compound which, proportionally, in experimental animals at least, could not escape detection. They conclude that the storage of oxygen is only temporary, occurring in muscular repose and used up during the balance of the day by muscular work. With the subject Breithaupt quotients of 0.63, 0.66, and 0.69 were obtained in duplicate experiments on the last three days of his 7-day fast and, immediately after, during work the quotients were 0.79, 0.77, and 0.74. The substance which fits these requirements obviously is glycogen and they believe it is formed in repose from body protein.[5]

Bernstein and Falta (15) had as a normal subject a man who for two days previous to their feeding experiment lived on a diet of vegetables and did heavy muscular work for the purpose of removing glycogen from the body. Then for three days on a limited diet containing only 300 gm. meat, 3 eggs and 50 gms. cheese he gave (by the Zuntz-Geppert method) R.Q.'s of 0.66, 0.668, and 0.684. They state that such quotients can only be explained by the formation and excretion of sugar or ketone bodies. No

[5] The authors do not attempt to justify this belief by calculation. Obviously, too, modern methods would require recovery allowances after muscular work.

sugar was present in the urine but a strong ferric chloride reaction was present and levo-rotation. The authors overlook the possibility of temporary storage of glycogen.

To Magnus-Levy (16) is due the credit of first stating clearly the limits of the relationship between oxygen intake and carbon dioxide elimination which are reached by maximal ketonuria in the diabetic. He originated the calculation which was later elaborated and made familiar to American readers by Lusk (17, 18). He states that for the diabetic to attain an R.Q. of 0.65 sugar must come from fat and an R.Q. of 0.60 could only be accounted for if in the metabolism of 200 gms. protein and 250 gms. fat, 300 gms. sugar were formed besides 40 gms. β-hydroxybutyric acid!

Andererseits sei darauf hingewiesen, dass under Umständen Respirationsversuche eine Bildung von Zucker aus Fett mit Sicherheit beweisen könnten . . . Freilich würden dazu nicht kurze Respirationsversuche ausreichen, sondern mindestens einen, am besten aber mehrere Tage, erstrecken.

Of course such conditions are impossible, and while the interpretation of short experiments is difficult, there is always the satisfaction of reproducibility under given conditions and the conviction that constant results must have definite causes. Much confidence in short-period experiments has been gained in 30 years, because technics are better understood as well as the errors which must be avoided.

Shaffer (19) has well stated that the general principles (which may be expected to produce low quotients) are the same in the normal and the diabetic, and Joslin (20) recognized ten years ago that these low quotients had been found by reliable technics far too often to be longer ignored. Among 113 patients (diabetes) studied by the Benedict "Universal" method between 1908 and 1917, R.Q.'s of 0.69 and below were observed in Joslin's work on 18 occasions with 9 patients in the post-absorptive state. Following a meal of C.35, P.10, and F.55, one patient gave successive quotients on the same day of 0.67, 0.65, 0.67, and 0.58! The next day and the next the same subject gave R.Q.'s as low as 0.62. A number of others showed individual quotients after food (the author does not state at what interval after food in any case!) of 0.67 and 0.68 and several had quotients of 0.68, 0.69, and 0.70 as an average of several periods in which "were included quotients higher than 0.71." Many others even more strikingly unusual could be cited from this work. Ketosis was not measured quantitatively, but the author finds that "acidosis was always accompanied by a falling respiratory quotient." With reference to glycosuria it was notable that amounts of sugar in the 24-hour urine of from 6 to 25

grams were necessary to produce any lowering effect on the R.Q.; amounts between 26 and 100 grams had very little additional effect; but "amounts over 100 grams imply that large quantities of fats are burning, accompanied usually by acidosis, and then the quotient is lowered further." In general a low R.Q. was accompanied by a high percentage of blood sugar and a high R.Q. by a significantly lower, though not necessarily normal, percentage. No direct relationship between blood fat and the R.Q. could be detected.

Wilder, Boothby, and Beeler (21) confirmed the occurrence of low quotients in a single diabetic patient studied over a long period by the Tissot-Haldane method. They found many very low post-absorptive quotients early in the morning—as low as 0.65 (non-protein) and often with very little sugar in the urine and very slight acidosis. Their table on p. 328 gives 0.65 as the average of 8 R.Q.'s following a meal of 85.5 grams fat. Table II on p. 331 shows several tests of the specific dynamic action of high fat meals of which the following are illustrative.

Basal R.Q.'s	June 1 0.69	June 2 0.68
Breakfast containing 34.9 gm. protein and 42.1 gm. fat		
After meal		
½ hr.	0.61	0.62
1 hr.	0.63	0.65
2 hrs.	0.65	0.65
3 hrs.	0.64	0.66

The authors state that they are certain these quotients were correctly obtained, but that "it is impossible, as Professor Lusk has emphasized, to make correct allowances for such factors as temporary alteration in the character of the respirations, so common in all respiration experiments, for variation in the CO_2-combining power of the blood, depending on whether acidosis is increasing or diminishing at the time of the test, and for utilization of oxygen and carbon dioxide in the formation of acetone bodies." They state also that this patient "tried so hard to be quiet and breathe normally that at times during the collection of expired air there was underventilation of the lungs, causing retention of CO_2." We take no exception to the statement that it is difficult to make proper allowances for disturbing factors. Nevertheless, it is necessary to make the attempt, particularly as regards the influence of acidosis. That is one of the major objects of

the present study. Those influences have been overestimated, as will presently be shown. As for underventilation of the lungs, it strikes the present writers as exceedingly unlikely that underventilation should recur at collection periods so regularly and in just the proper variation of degree to produce that particular sequence of quotients which, according to the present hypothesis, would be produced by formation of oxygen-rich material from oxygen-poor, and the retention and subsequent oxidation, or partial oxidation, thereof. It is one of the advantages of the Benedict method, carried out with a recording spirometer, that one can be sure of the normal depth of respiratory movements.

Neither Joslin nor Wilder, Boothby, and Beeler attempted a theoretical discussion of the limits of their low quotients in relation to the characteristics of metabolism. That service was rendered by Shaffer (19) in one of his discussions of anti-ketogenesis. He found that a total R.Q. of 0.76 indicated the oxidation of a metabolic mixture made up approximately of equimolecular amounts of ketogenic substances (fatty acids or ketogenic amino acids) and of antiketogenic derivatives of amino acids, glycerol, or carbohydrate, expressed as total glucose. A quotient of 0.73 indicated 2 mols. of ketogenic to 1 of antiketogenic substance, while one of 0.80 indicated 0.5 mol. of keto to 1 of antiketogenic glucose equivalent. Later Shaffer (22) modified the rigidity of these proportions by admitting that some subjects undoubtedly show small but definitely abnormal amounts of acetone when mixtures of metabolites, judged by the R.Q.'s or by calculation, correspond to a ketogenic:total glucose ratio of about 1:1; while other subjects may have a ratio of about 2:1 before large amounts of acetone bodies appear in the urine.

Baer (23) long ago noted that different mammalian species react differently to withdrawal of carbohydrate. While man and monkey react with acidosis on mere withdrawal, the swine exhibits it only in complete starvation, the dog, goat, and rabbit only when poisoned with phlorhizin.

Lyon, Dunlop, and Stewart (24) recently have found many low quotients in obese patients taken in the post-absorptive condition. Out of 15 determinations on subjects whose reducing diet was low in carbohydrate and contained approximately 1000 calories the quotient was below 0.70 in 12, or 80 per cent, of the tests. Such quotients occurred on higher caloric intakes, but much less frequently. Every possible precaution seems to have been taken. Their computation of maximum possible yield of carbohydrate from protein and glycerol confirms our own, made before their contribution appeared (see p. 549).

TABLE VI

AVERAGE ACETONE PRODUCTION ON DIFFERENT FAT LEVELS—SUBJECT J (MALE)

Date 1930	F.A.:G Ratio	Basal	3 hrs.	6 hrs.	9 hrs.
Total Urinary Acetone, Mgms./hr.					
Jan.	1.5:1	Negative to ferric chloride			
Jan.	3.0:1	25	57	70	24
Feb.	4.1:1	40	63	61	45
Total Blood Acetone Mgms./100 cc.					
Jan.	1.5:1	9	7	8	—
Jan.	3.0:1	16	13	14	19
Feb.	4.1:1	24	40	40	32
ADAPTATION TO FAT—SUBJECT H (FEMALE)					
Urine—Total Acetone, Mgm./hr.					
Jan.	1.5:1	11	—	—	—
Jan.	3.0:1	171	274	353	364
Feb.	4.1:1	324	—	—	350
Apr.	1.5:1	18	18	20	—
May	4.1:1	187	216	206	—
Blood—Total Acetone, Mgm./100 cc.					
Jan.	1.5:1	42	—	40	—
Jan.	3.0:1	55	52	51	65
Feb.	4.1:1	58	43	51	41
Apr.	1.5:1	14	8	14	—
May	4.1:1	29	39	27	25

No established fact in the literature, it is believed, directly contradicts the hypothesis that low respiratory quotients reliably obtained under the conditions described on page 524 may denote some conversion of fat to carbohydrate. Periods of activity alternating with periods of repose in fasting, as in Lehmann and Zuntz's work, depletion of glycogen followed by a meal containing little or no carbohydrate but much fat in normals as in Bernstein and Falta's experiments, heavy fat feeding to diabetics as in Joslin's and Wilder, Boothby, and Beeler's studies, low carbohydrate, low calorie diets for the obese, have demonstrated many low quotients. We believe systematic attention to the conditions we have described as essential would demonstrate many more. The effects of acidosis will not furnish an

answer to the question of the *possibility* of gluconeogenesis from fat as will be seen below.

Relation of actual R.Q.'s to ketosis.—That the degree of acidosis bears a direct relationship *to the amount of fat fed* was to be expected, and, with allowance made for the phenomenon of adaptation, is perfectly evident in these experiments, as may be seen from Table VI which summarizes results for subjects J and H. Respiratory quotients,[6] however, were not found to depend on the amount of fat in the diet but rather upon the condition of the subject at the time the fat was taken. A person with a very high natural tolerance or a recently acquired tolerance for high fat was apparently not so good a subject for producing low R.Q.'s as a person with low tolerance.

Chart 5 exhibits the wide scatter which one obtains in attempting to show a relationship between R.Q.'s and the ketosis as represented by "total acetone" of the blood. Three subjects, H, J, and M furnish the data, but not at all equally. Some of the "points" represent averages of as many as six different amounts of total acetone for a single R.Q. Plotting all the individual points only increases the scatter. The chart therefore represents the most conservative, legitimate use of the data. When a similar chart containing different symbols for each of the three subjects was prepared, there was no grouping characteristic of each subject. Examination of Tables I to V has already confirmed this impression.

Chart 6 exhibits an even wider scatter concerning the relationship between the ketonuria and R.Q. In this instance, however, there is a tendency toward an individual grouping. All of the "points" above 150 mgm. "total acetone" per hour were contributed by subject H, but by no means all of the points contributed by this subject lie in this range. Of all the points below 100 mgm. 11 were contributed by subject J, 12 by subject M, and 4 by subject H. The quotients corresponding to these points range from 0.65 to 0.77. The chart gives a very faint impression that a much larger collection of data would exhibit a broad drift of coördinate points from the upper left to the lower right hand corner of the field. Even so, the scatter would be quite too wide to denote anything like a close correlation. The conclusion from these two charts inevitably is, that the R.Q. is not intimately dependent upon the ketosis, as judged by blood analysis or excretion. The only way in which ketosis could be linked with the quality

[6] In these experiments where the protein metabolism was so small compared with the total, no material difference results whether total R.Q.'s or non protein are used. All quotients unless otherwise specified are total.

of the respiratory metabolism in these experiments would be to suppose that following the test meal there must be a considerable accumulation of ketone bodies in certain tissues before they appear even in the blood.

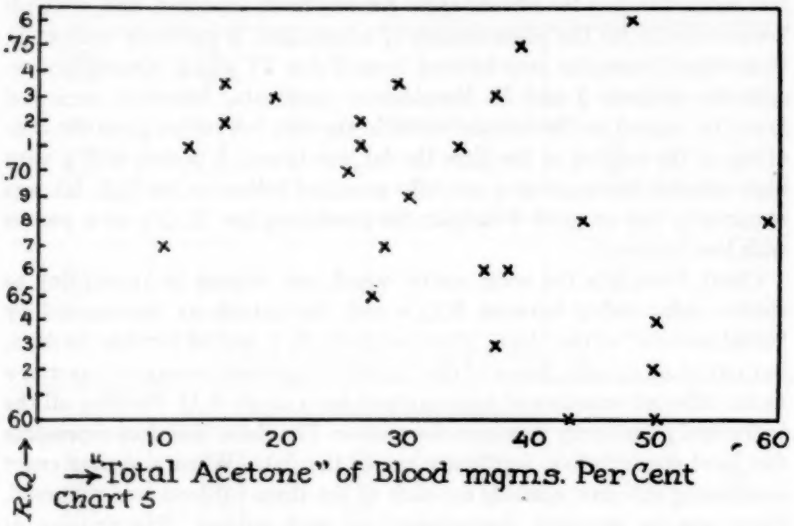


Chart 5

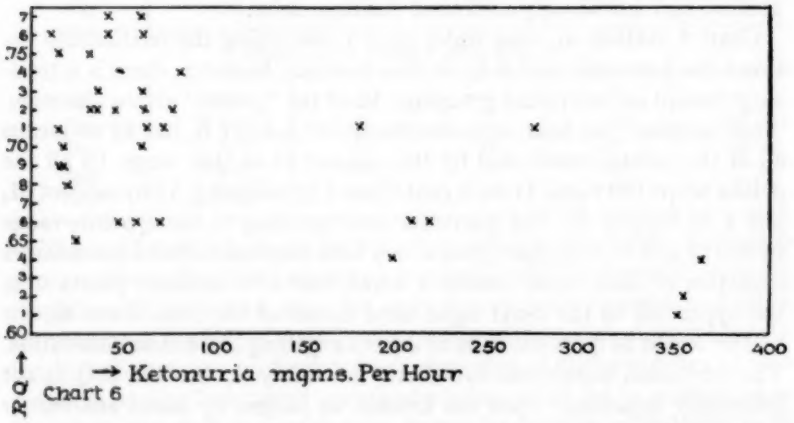


Chart 6

Relation of R.Q. to protein metabolism.—Lehmann and Zuntz surmised that low quotients in their fasting subject might be due to the formation of glycogen from protein. By analogy with the formation of sugar from protein in diabetes, this suggestion strikes one as very plausible. The ques-

tion is, how much of a depression below fasting level could be accounted for in this way. Fortunately we have a time-honored, though not necessarily unobjectionable, method of calculation which may be used to gain an idea of the effect. It is employed here for want of a better. Magnus-Levy (16) originated the method and it has been employed by Loewy, by Frentzel and Schreuer, Geelmuyden, Lusk, and others. Before putting it to a test we must select a period and translate the metabolism as given in calories per hour into grams of fat and protein. The heat production for quotients below 0.707 in previous tables has been calculated as if the quotient were actually at this level. There is as yet no widely accepted table of heat values of oxygen for non-protein quotients below this. Bernstein and Falta (15) prepared by extrapolation from the Zuntz and Schumburgh standards a table of values down to 0.60. This will be used *for our purpose now* with the conviction only that it is the lesser of two fallacies. In a later paper it is hoped to give more exact values from direct calorimetry.

Selecting the period from 3rd to 6th hour after food on April 8 for subject H (Table I) we find that 0.436 gram N per hour was excreted. The heat value of this metabolism would be 11.55 Calories, the non-protein R.Q. works out at 0.65, the calories from fat by Bernstein and Falta's table would be 46.53 Cal. and the grams fat metabolized in the hour 4.897 at a caloric value of 9.5 per gram.

Assuming an availability of 58.5 per cent (D:N, 3.65) the protein metabolized would yield 1.592 grams sugar. This amount of sugar, however, would make only 1.434 gm. glycogen. The effect of this glycogen formation and the substitution of an isocaloric quantity of fat in combustion, may be shown in the conventional manner as follows:

	C	H	O
1 gm. N-free protein contains,	0.415 gm.	0.044 gm.	0.0769 gm.
2.725 gm. N-free protein (N×6.25) contains,	1.130	0.1199	0.2092
1.434 gm. glycogen contains	0.637	0.0889	0.7084
Leaving	0.493	0.0310	−0.4992
0.638 gm. fat, isocaloric for glycogen,	0.488	0.0759	0.0733
Making a total of	0.918	0.1069	−0.4159
Adding for 4.897 gm. fat (see above)	3.746	0.5828	0.5632
Total	4.727	0.6897	0.1473

Deducting for intramolecular H_2O		0.0184	0.1473
Remaining to be oxidized,	4.727	0.6713	0.0

Requiring 17.943 gm. O_2 and producing 17.301 gm. CO_2
In liters, 12.550 L. O_2 and 8.823 L. CO_2
R. Q. = 0.703

The R.Q. for the protein and fat metabolism as calculated from the actual data by the same sort of procedure as above, but omitting the data for glycogen and the substituted fat, would be as follows:

	C	H	O
1 gm. N-free protein contains	0.415 gm.	0.044 gm.	0.0769 gm.
2.725 gm. N-free protein contains	1.130	0.1199	0.2092
4.897 gm. fat protein contains	3.746	0.5828	0.5623
Total	4.876	0.7027	0.7715
Deducting intramolecular H_2O,		0.0957	0.7715
Remaining to be oxidized,	4.876	0.607	0.0

Requiring 17.826 gm. O_2, producing 17.846 gm. CO_2
In liters 12.474 L. O_2 producing 9.086 L. CO_2
R.Q. = 0.728

The effect of the formation of glycogen and the substitution of an isocaloric quantity of fat in combustion would be to reduce the R.Q. from 0.728 to 0.703, a depression of 0.025. This particular period represents the greatest ratio of protein metabolism to the non-protein metabolism observed for subject H. The correction therefore is maximal.

The period which revealed the lowest protein metabolism in relation to the non-protein for this subject is the last period of February 2 (Table III) where the N output per hour was 0.163 gm. The same calculation for this period shows that formation of glycogen and substitution of fat isocalorically for the fuel thus lost would depress the R.Q. exactly .01. The correction from this cause may therefore be placed at not less than .01 nor more than .025. Examination of the data for the other subjects for whom the protein metabolism was known has convinced us that the range of correction for them would come within the same limits. In the particular periods chosen for illustration the correction for the larger protein metabolism would raise the observed R.Q. from 0.68 to 0.705; for the lower protein metabolism it would raise the observed R.Q. of 0.61 to 0.62! It is obvious that glycogen formation from protein cannot account for the lowest quotients obtained with subject H, but that it might account

for a slight depression. Obviously, too, the combustion later of glycogen from this source would account for a rise of quotient above the theoretical. (See also the computation of Lyon, Dunlop, and Stewart.)

Meantime there is to be considered a combined correction for ketosis and alteration of protein metabolism occasioned thereby.

Correction for ketosis and ammonia formation.—There is no reason from the data of this paper, or from the literature, to suppose that a correction to the R.Q. for the degree of ketosis found would be significant. Nevertheless to make all the corrections possible it has been carried through. The general method is familiar and can be found in Lusk's "Science of Nutrition," 4th edition, p. 671. In the present instance the correction has been made on the basis of the increase of the acetone bodies in the blood during the three-hour period within which the respiration period lies. Acetone and diacetic acid have been treated as one quantity, β-hydroxybutyric acid as another. In order to make the correction very generous the quantities in the blood have been assumed as applying to the *entire weight of the body*. The result is to raise the non-protein R.Q. by an amount from .007 to .02 in the three instances shown in Table VII. Actually the increase would not be more than 1/5 of this amount, even allowing for some formed ketones which have not appeared in the blood! Magnus-Levy calculated that a formation of 40 grams β-hydroxybutyric acid would lower the R.Q. only .012; correction for this large formation therefore would raise it only by a like amount.

Against this stands the correction for production and excretion of ammonia instead of urea, used in neutralizing the ketonuria. In Table VII also are found the changes in non-protein R.Q. occasioned by correction for the diminished oxygen absorption caused by shunting off NH_3 before it is changed to urea. The R.Q. is higher because of ammonia excretion; the correction therefore lowers it by the same amount. For the largest amount of ammonia shown (subject H for Feb. 2) the correction is .01 downward. For the least amount shown it is negligible. The net change for both corrections is found by comparing the first non-protein R.Q. in Table VII with the last.

Conversion of glycerol to sugar.—It is generally agreed that in diabetes sugar can be formed from glycerol. The simplest equation is, $2C_3H_8O_3+O=C_6H_{12}O_6+H_2O$, i.e., dehydrogenation. Should the same take place in the normal subject what effect would be produced on the R.Q? The attempt to answer this question has been made experimentally. Glycerol in amount equivalent to the theoretical total yield of the fat ingested on an adjacent

day was given to two different subjects. They reacted quite differently, as may be seen from Chart 4. Subject M was studied by both the Benedict and the Tissot-Haldane methods on October 1 when glycerol was taken and on October 2 when the fat was ingested. Previous to the glycerol day were 3 days of high-fat (all cream) diet. With only one exception on each day the R.Q.'s taken one-half hour apart by the two methods, agreed remarkably well. Comparing the Tissot results on the two days because they are more complete, they are, with the exception of the first hour period as nearly duplicate results as one could ever hope to get on two successive days following identical meals. There was, however, no early drop in the R.Q. because, on the present hypothesis, there was no demand for glycogen at this time. Why there was no demand may be explained by the phenomenon of adaptation. Subject M had been through the high-fat experiment in May preceding and at that time exhibited some signs of adaptation (See Table V) to the diet, not connected with ketosis. As with subject H, on resuming the diet several months later better tolerance was shown. It is useless at present to speculate on just what all is implied in better tolerance. The manifestation of it for present interest is the nearly uniform combustion qualitatively throughout the experimental day. It is astonishing that with a meal furnishing so little energy as did the glycerol, as compared with the fat meal, the metabolism could be so nearly the same qualitatively. Quantitatively the two days were quite dissimilar. Instead of a large dynamic effect which is obtained regularly from the cream diet, there was on the glycerol diet an actual depression of heat production which continued for many hours. But that difference need not detain us at present.

Subject F was studied by the Tissot method only. After three days of the high-fat (ratio 4.1:1) diet the subject took a test meal consisting of 665 grams 4X cream, containing approximately 249 grams fat, and the respiratory metabolism was followed for six hours thereafter in hourly periods. On the fifth day the meal consisted of 26.6 grams glycerol. The R.Q. curves are shown in Chart 4. The agreement is not quite so good as on the two days for subject M, but the early fall and subsequent rise in the quotients are striking and the average of the first four periods, following the first low quotient, is the same, 0.74, on the two days, showing that the recovery was on the average the same, so far as they could be compared.

Both experiments are strikingly suggestive of an identity of effect from glycerol as such and from glycerol included in the fat. It would seem that we have in the conversion of glycerol to sugar (glycogen) an adequate

TABLE VII
CORRECTION OF NON-PROTEIN R.Q. FOR NH_3 AND ACETONE BODIES

Subject	Urine N	Non-prot. R.Q.	Urine NH_3N	Corrected non-prot. R.Q. for NH_3N	Acetone + diacetic gm.	β-hydroxy-butyric gm.	Gms. blood incr. or decr.		Corr. non-prot. R.Q. for ketosis
							Acetone diacetic	+ β-hydroxy-butyric	
H	0.163	0.70	0.049	0.69	0.72	0.278	0.0	+0.2	0.702
J	0.222	0.67	0.018	0.67	0.011	0.047	0.33	+4.35	0.69
M	0.372	0.724	0.036	0.722	0.012	0.081	+7.0	+1.66	0.729

explanation for the low quotients. In the experiment on subject F, where glycogen was needed, and in subject M where it was not needed, the glycerol was metabolized with body fat just as it was with the cream fat.

The effect on the R.Q. of the conversion of glycerol to glucose may be pictured with substantial accuracy as follows. Arguing that the glycerol given as such to subject F was completely converted to sugar within 3 hours and that 2/5 of the transformation occupied the first hour, 2/5 the second and 1/5 the third,[7] there would be 10.64 (2/5 of 26.6) grams available the first hour. The requirement for oxygen would be $(10.64 \times 16/184) = 0.925$ gms. or 0.647 liter. Subtracted from the oxygen absorbed that hour (20.6 liters) this would leave 19.95 liters which, divided into the CO_2 production (13.6 liters), would give a quotient of 0.682 instead of 0.660, a difference of .022. Probably this is a maximal correction from this cause. Assuming conversion at the same rate on the high fat day, the correction would be only .015 because of the much greater oxygen absorption occasioned by the specific dynamic action. The sequence of the R.Q.'s on this day indicates that the conversion did not proceed quite so rapidly or so far as on the glycerol day. In any event it is clear that the conversion of glycerol to sugar (glycogen) would need to proceed even more rapidly than estimated above to account for the sagging of the quoteint within the first hour after absorption. *One has difficulty also in picturing how glycerol can be split off for glycogen formation faster than fat is burned;* for so long as the fat is in transport or in deposit the glycerol is required to keep it neutral. The largest

[7] These fractions are based on the increases in oxygen absorption.

hourly combustion recorded in this study is 115.5 Cal. After deducting for protein metabolism, the balance is accounted for by combusion of 10 grams fat. Ten per cent of this would be glycerol. The effect of the conversion of 1 gm. of glycerol to glucose would be negligible in such a total combustion.

Combined corrections.—Should conditions ever arise in which glycogen would be demanded from the protein metabolism and the glycerol of the fat metabolism simultaneously, this combined conversion at maximal intensity would not account for a depression of more than .03 in any hourly period of this study. Adding the largest actual net correction imaginable from the two phases of ketosis, the total effect could not be more than .04. If the normal R.Q. for the all-cream diet be taken at 0.73,[8] then any R.Q. below 0.69 in these experiments calls for some other explanation. There are far too many of that class to be charged to errors of technic or undetectable alterations of respiratory movements. They have been obtained by both Tissot-Haldane and Benedict methods. On the same day (February 2) within one-half hour of each other by identical technic on the same Benedict machine, one subject (H) gave an R.Q. of 0.60 and the other (J) 0.75. Subject H gave by far the largest number of low R.Q's and there was at the same time plenty of evidence that she could not properly burn the fat—low specific dynamic action, marked ketosis, etc. Make all the corrections possible for glycogen formation and ketosis (they rarely go together) and the R.Q.'s still are far below normal for combustion of fat.

Other possible use for oxygen.—It is not necessary to conclude hastily that fatty acids go to sugar as readily as sugar goes to fatty acids, as many young authors, trained to regard all reactions as reversible, are inclined to do. If that were true it would not be possible to render dogs and cats glycogen-free on a cream diet, as Dale's laboratory[9] claims to have done! There is one other possible use for excess oxygen which we have not seen mentioned in the literature. On the modern theory of beta-oxidation of fats there is a clear possibility that a considerable amount of oxygen may be employed in the formation of intermediary acid radicals and other groupings before any carbon dioxide production gets well started. With an enormous flood of fat entering the circulation, it would seem that intermediary partially oxidized bodies not yet small enough to be water-soluble and excretable by the kidney would account for enough oxygen to lower

[8] This would be a minimal average, allowing for protein metabolism and a technical error of .02.
[9] See paper by Gregg, D. E., *Amer. Jour. Physiol.*, 1933, 103, 79.

the R.Q. for several hours, possibly all day. Later, perhaps when the antiketogenic effect of glycerol in larger amount becomes available the oxidation quickly proceeds to the end and there is a larger elimination of CO_2. Several sequences in the tables suggest this. The initial drop in R.Q. is due more often to a rise of O_2 than to a change in CO_2; later when the R.Q. rises, not only is the oxygen falling but the CO_2 is rising. In this view of the matter it would not be the glycogen hunger alone but the lack of catalysts (oxidases?) perhaps which would establish the condition precedent for low quotients following high fat.

The conception here advanced is not unlike that of Geelmuyden (25) in that the intermediary substances would contain more oxygen (or less hydrogen); but differs from Geelmuyden's ideas in that the desaturation would not need to proceed to the carbohydrate stage to account for the quotients alone. The theory here advanced will be discussed in a later paper.

The authors do not claim to have proved the conversion of fat to carbohydrate in the human body. They think the low quotients are a reality; they feel certain that neither glycogen formation from protein and glycerol nor ketosis, nor both can, in these experiments, account for quotients lower, on the average, than 0.69. If it could be shown that no other use of extra oxygen were possible, or if the sugar supposed to be formed from fat involving these low quotients could be found, the proof in either case would be logical and complete. It is the authors' conviction that the extra sugar from fat never has been clearly demonstrated. Logic compels us to wait for further evidence.

Summary and Conclusions

1. Respiratory metabolism studies on seven different subjects taking high-fat diets, following meals containing varying amounts of butter fat, show many respiratory quotients below the theoretical level for oxidation of fat.

2. The occurrence of these low quotients does not depend upon the amount of fat taken in the experimental meal, nor upon the F.A.:G ratio of the general diet, so much as upon the tolerance of the subject.

3. Adaptation to or tolerance of high fat in the sense of better capacity to oxidize fat and producing less ketosis may be acquired and be retained for several months.

4. The level of the respiratory quotient bears no intimate relationship to the demonstrable ketosis or ketonuria.

5. The hypothesis with which the work was undertaken, calling for a

special sequence of low quotients soon after the test meal followed by higher quotients later, has been realized in a number of individual experiments, but not in all. The most successful from this point of view are the early experiments for each subject performed before increased tolerance had developed. Low quotients may be induced to appear also by shivering at a time when, ordinarily, they would be high.

6. Production of glycogen from the protein metabolism could account for a depression of the R.Q. at most of .025; while production of glycogen from glycerol, assuming that only the glycerol of the fat metabolism were available, would produce a depression of not more than .003. Correction of the quotient for the demonstrable ketosis and consequent ammonia formation would not account for more than .005. At most the combined effect of all these factors would not account for quotients lower than 0.69.

7. The formation of glycogen from fat (beyond the amount which could arise from glycerol) having never been proved, it would be premature to conclude that the quotients below 0.69 in this work demonstrate gluconeogenesis from fatty acids.

8. It is suggested, as an alternative explanation, that in the oxidation of fatty acid chains the uptake of oxygen may considerably outrun for a time the production of carbon dioxide and thus account for a depression of the R.Q. A process of desaturation which would remove hydrogen, but not produce any carbon dioxide, followed by oxidation with production of CO_2, would fulfil the requirements.

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Editorial Review

OTHER FACTORS THAN VITAMINS B AND G IN THE VITAMIN B COMPLEX*

OUR knowledge concerning the multiplicity of factors in the vitamin B complex is constantly undergoing revision as a result of an ever-increasing number of contributions to this field of vitamin research. The accumulation of evidence forces the most conservative to conclude that not only one but probably several components exist besides the two better defined factors, the antineuritic B(B_1) and the antipellagric G(B_2). Our uncertainty as to the nature of these additional components may be well emphasized by quoting from the recent review of vitamin B by Kruse and McCollum (28). They state, "Of the seven components at present advanced as constituents of the water-soluble B complex, the pigeon needs three, and the rat requires five for optimum nutrition. Thus far only one component, the antineuritic vitamin B, has been shown to be definitely needed in common by the two species; on the other hand, there is some evidence that certain components are required by one species and not by the other. The entire question of the nutritive needs of the pigeon and the rat for growth is in a transitional state, and judgment concerning it may well be reserved. It is not impossible that the estimated number of B components may be reduced by the demonstration that certain of them are identical. However, the available evidence that the pigeon and the rat require different components of the B complex for maximum weight is of such convincing character that it cannot be dismissed."

This newer knowledge concerning the "ultra-complexity" of the vitamin B complex will be far reaching in its effect on vitamin studies. Radical changes must be instituted in the methods used for the study of any single one of the components of the vitamin B complex. Much of the work that has been done in the past on vitamins B and G is subject to criticism on account of failure to insure the presence of all components, other than the one being studied, in the diets employed. Quoting again from the article of Kruse and McCollum (28) on the antineuritic factor, "Certainly there is abundant opportunity for error in attempting to correlate the pathologic and physiologic manifestations of the rat and the pigeon on a defective diet: unless the ration of each species is made adequate with respect

* The material contained in this review was taken from a paper read before the Biological Division of the American Chemical Society at the Denver meeting in August, 1932, as part of a symposium on the so-called "third" factors of the vitamin B complex.

to all factors required by it, with the exception of the antineuritic component, the observations may not be comparable." What is true of the antineuritic factor B is equally applicable to G or to any other component of the vitamin B complex. It is obvious that in the future more care must be taken in the preparation of basal diets for the study of the better defined factors, B and G, as well as of the newer allegedly existing components.

The present attempt to review the literature on the so-called "third" factors of the vitamin B complex seems particularly appropriate, inasmuch as there are only two such surveys that might be considered in any sense comprehensive. The first by Peters (32) was presented two years ago and only two years after the first paper on the "tripartite" nature of vitamin B appeared; the second by Harris (21) is contained in a review of recent literature on all of the vitamins and, although it contains a valuable list of contributions to the literature on the subject, "exigencies of space have excluded a discussion seriatim of the separate factors." Although both articles are well worth the attention of workers in this field, as well as that of others less directly interested, a comprehensive review of the literature at this time is quite opportune when one realizes that a considerable number of papers have appeared since Peters' review.

The difficulty of any effort to correlate the results of the different workers in this field needs no great emphasis. However, when one considers that the methods employed by different workers vary tremendously, that the evidence is oftentimes presumptive and based upon feeding experiments where sub-normal growth has been observed with diets supposedly adequate in all of the known factors, that very few of the workers have attempted to fractionate the factors from a single source rich in all of the components of the vitamin B complex, and that the evidence of new factors is not based upon the production of characteristic lesions due to the supposedly "new" vitamin deficiency, one approaches a critical analysis of the subject with considerable temerity. Especially is this true when one realizes the validity of the recent statement of Harris (21) that "any direct comparison or collation of results is rendered almost impossible by the fact that widely diverging definitions and basal assumptions are taken by the different investigators." The review of the literature that follows is made with a full realization of these attendant difficulties.

Vitamin B_1

In 1927 Williams and Waterman (51, 52) reported that pigeons, brought to a subnormal weight level on a synthetic diet free from vitamin B com-

plex or on a diet of polished rice, required for weight restoration some other factor than the antineuritic vitamin B and the antipellagric vitamin G. The basis of their claim was the fact that, although the decline in weight on such deficient diets could be stopped by the addition of a special activated fullers' earth preparation of the antineuritic factor from yeast, restoration of weight failed to occur even when autoclaved yeast as a source of vitamin G was given as a further supplement, but did take place upon the addition of air-dried brewer's yeast. Apparently, the recovery in weight, and the accompanying improvement in general condition, must have resulted from the presence of a thermolabile factor in the yeast other than the antineuritic vitamin B. Whole wheat was also shown to contain this "new" factor. Furthermore, it was demonstrated that rats do not require this second thermolabile component of the vitamin B complex. This factor has been designated vitamin B_3 by Williams and Eddy (50).

Randoin and Lecoq (34) had shown in 1926 that pigeons must receive, in addition to the antineuritic vitamin, a growth-promoting or nutritional factor (*"vitamine d'entretien ou de fonctionnement"* or *"vitamine d'utilisation nutritive"*), and that certain yeasts or yeast extracts prevented loss of weight but not polyneuritis in adult pigeons. In the following year Lecoq (29) claimed that at least three factors in the vitamin B complex, the antineuritic factor, a thermo-stable, alkaline-stable, growth-promoting factor, and the "nutritional factor" of Randoin and Lecoq, are needed by the pigeon. As Randoin and Lecoq had demonstrated that their nutritional factor was destroyed by autoclaving in an alkaline medium, Lecoq (30), in discussing the question of the identity of the nutritional factor of Randoin and Lecoq and the Williams and Waterman vitamin B_3, expressed the opinion that they are the same.

The requirement of pigeons for the Williams and Waterman vitamin B_3 has been further confirmed by Peters (32) and by Eddy, Gurin, and Keresztesy (12). The latter investigators have shown that this vitamin B factor is also needed for normal growth by chicks, even when fully supplied with vitamins B and G. They have demonstrated that vitamin B_3 is even less stable to heat than vitamin B, especially in alkaline solution, thereby confirming the original report of Williams and Waterman that their factor was exceedingly thermolabile.

Vitamin B_4

In 1929, Reader (36) reported evidence for the existence of a third factor in the vitamin B complex. Addition of vitamin B concentrates, prepared according to Kinnersley and Peters (27), and of vitamin G, as al-

kaline autoclaved yeast extract, to a basal diet deficient in the vitamin B complex did not induce normal growth of rats even when the amounts of B and G were increased above the minimum requirement. Growth either ceased or proceeded at a very subnormal rate. The substitution of marmite (whole yeast extract) for the B and G resulted in a resumption of growth to maximum weight. This indicated that yeast contains, in addition to B and G, a third factor necessary for normal growth of the rat. This third component was shown to be very thermolabile, much more so than vitamin B. In a later paper, Reader (37) reported the preparation of a concentrate of this factor from an extract of the mercuric sulphate precipitate formed in the process of Kinnersley and Peters (27) for the preparation of vitamin B. Experiments with this concentrate completely confirmed the earlier work on the existence of a second thermolabile rat factor. Although she had tentatively adopted the term vitamin B_3 for this rat factor, Reader (38) finally suggested that vitamin B_4 be used to denote the third rat B factor, thus avoiding confusion with the Williams and Waterman thermolabile pigeon factor which had been termed vitamin B_3.

Soon after Reader's first paper, Chick and Roscoe (8) questioned whether the results obtained by Reader were due to a deficiency of a new vitamin, and suggested that they might have been caused by an insufficiency of vitamin G, a portion of which had been destroyed by the alkaline autoclaving used in the preparation of the vitamin B_4 supplement by Reader. Later, however, Roscoe (40), as a result of having observed in negative control rats the listlessness, hunched attitude, and inflamed and swollen paws typical of vitamin B_4 deficiency as described by Reader, admitted the existence of vitamin B_4 and gave evidence for its distribution in vegetables. Halliday, Nunn, and Fisher (20) have recently described a thermolabile "third" factor necessary for the growth of rats that seems to be identical with the vitamin B_4 of Reader.

Vitamin B_5

In his Harben lectures, Peters (32) gave evidence that pigeons require another factor of the vitamin B complex in addition to vitamins B and B_3. Whereas preparations of these two vitamins were not sufficient as supplements to a diet deficient in the vitamin B complex to maintain the weight of pigeons, this was accomplished with fresh marmite (yeast extract) which lacks vitamin B_3 (Williams and Waterman factor). This new B component was shown to be destroyed by alkaline autoclaving, and Peters expressed the belief that it might be identical with the rat factor B_4

of Reader. Later, however, Carter, Kinnersley, and Peters (4, 5) concluded that this maintenance factor for pigeons was different from the rat factor B_4 of Reader, since it was heat-stable to an alkaline heating that would destroy vitamins B and B_4. Furthermore, they advanced other arguments against the identity of their maintenance factor, which they termed vitamin B_5, and the Williams and Waterman vitamin B_3.

As mentioned earlier in this paper, Randoin and Lecoq (35) demonstrated the requirement by pigeons of a thermostable, alkaline-stable, growth-promoting factor in yeast. Although they believed that this factor was identical with the antipellagric vitamin G, the fact that this latter vitamin is now generally considered not to be a requirement of pigeons makes it seem probable that they were dealing with a component identical with Peter's vitamin B_5.

"Factor Y" of Chick and Co-workers

Chick and Roscoe (7) reported that rats fed a diet in which egg white was the sole source of the vitamin B complex failed to grow even when vitamin B was added as Peter's antineuritic concentrate, whereas rats on autoclaved yeast and B grew normally. As they had demonstrated that egg white was a rich source of vitamin G free from vitamin B, the presence of a thermostable "third" factor in the vitamin B complex was suggested. Further evidence for the presence of this thermostable factor in yeast was given by Roscoe (39) and by Chick and Copping (6). In the latter paper it was shown that yeast extract, autoclaved under conditions sufficient to destroy completely its G content (4 hours; 120–125°; pH. *ca.* 10) still retained this new factor, which they designated as "Factor Y."

They emphasized the fact that Factor Y differs from the B_3 of Williams and Waterman and the B_4 of Reader in its stability to heat and alkali. As their paper appeared simultaneously with that of Carter, Kinnersley, and Peters (5), the question of the identity of "Factor Y" and vitamin B_5 was not raised. Although the requirement of pigeons for Factor Y and that of rats for vitamin B_5 have not been studied, the similarity in their stability to heat under alkaline conditions suggests the possibility that we are here dealing with one very thermostable factor which is required both by the rat and by the pigeon. Further investigation is necessary to clarify this point.

"Third" Factor of Coward, Key, Morgan, and Co-workers

In 1929 Coward, Key, and Morgan (10) reported that "light-white casein" contains a growth-promoting rat factor different from any of the

"recognized" vitamins and that it is not present in "vitamin free-casein".[1] Although unsuccessful in extracting this factor from yeast, they were able to obtain active alcohol and ether extracts of it from wheat embryo. The "casein" factor was destroyed by heat, at least partially. Coward, Key, Morgan, and Cambden (11) gave further evidence for the presence of a "new" growth-promoting factor in "light-white casein" (B.D.H.) and, subsequently, Coward, Key, Dyer, and Morgan (9) reported that all attempts by them to extract this growth factor from "light-white casein" had been unsuccessful. Furthermore, they demonstrated that the heating of "light-white casein" by Messrs. Glaxo in the preparation of their "vitamin-free casein" only partially destroyed this factor.

In this connection the work of Palmer and Kennedy (31) should be mentioned. They demonstrated that impure casein contains a factor other than antineuritic vitamin B that is necessary for growing rats. Later Kennedy and Palmer (26) expressed the opinion that this factor was vitamin G. There is still some possibility that they were dealing with the factor reported by Coward and co-workers. Williams and Eddy (50) also reported the presence of a thermolabile factor present in casein and brewers' yeast and necessary for the growth of rats. This factor could not be extracted from casein and in this respect and in its thermolability resembled the "casein" factor of Coward and co-workers. In 1919 Emmett and Luros (13, 14) had demonstrated that milk contains a water-soluble, heat-labile, growth-promoting vitamin other than vitamin B. Similar conclusions have been drawn by Supplee, Kahlenberg, and Flanigan (46). Whether the heat-labile factors reported by these two groups of workers will prove to be identical with that of Coward, Key, Morgan, and co-workers, future work only can determine. At present, it is impossible to judge whether the "casein" factor of Coward is identical with any of the B factors previously mentioned in this review.

"Third" Factor of Hunt

Hunt (23, 24) demonstrated the presence of a "third" factor necessary for the growth of rats and present in the residue remaining after autolyzed yeast has been extracted with weak acetic acid (0.01 per cent) and also in the fullers' earth residue obtained by treatment of the yeast extract with fullers' earth and by its subsequent extraction with concentrated barium hydroxide. Both of these residues when added to B and G fractions obtained from the same yeast gave increased growth as compared to that

[1] Glaxo.

obtained on the B and G fractions. Hunt suggested that the presence of the "new" factor in the fullers' earth residue might be explained by the supposition that some of the yeast residue remains in suspension in the water extract and is then adsorbed on the fullers' earth, remaining there after the extraction of the earth with barium hydroxide. He showed that the "third" factor was thermostable, as it was not destroyed when the yeast residue was autoclaved. In a subsequent paper Hunt and Wilder (25), using a stronger acetic acid (0.1 per cent) for extraction and extracting thus fourteen times, obtained the "third" factor not only in the yeast residue but in the non-adsorbable fraction after treatment with fullers' earth. Further investigation of its stability to heat demonstrated that it was stable when heated in acid medium but thermolabile in alkaline medium (pH 9). Also it was shown that the "third" factor supplements vitamin G in the prevention and cure of pellagra. The fact that the "third" factor is soluble, at least to some extent, in water and thermolabile in alkaline medium suggests its possible identity with Reader's vitamin B_4. However, further work must be done to prove or disprove this hypothesis.

"New" Nutritional Factors of Stiebeling

As the result of an observed variability of symptoms of G-avitaminosis in their experimental animals on a diet supposedly deficient in vitamin G but adequate in other respects, Sherman and Sandels (44) raised the question of the possible multiple nature of vitamin G. Subsequently, and quite recently, Stiebeling (45), working in Sherman's laboratory, found that, when skim milk powder was fractionated and then fed to rats on a diet deficient only in vitamin G, better growth was obtained with the combined fractions than when double portions of either single fraction were fed. A similar observation was made when the experiment was repeated with fractions of skim milk powder prepared by a second procedure. As an explanation of these experimental findings, Stiebeling postulated the presence in the milk powder of a "new" nutritional factor necessary for rats. In support of this hypothesis, she presented a second type of evidence. When rats with an unfavorable nutritional history were given graduated portions of milk as a source of vitamin G in the Bourquin and Sherman method of assay of this vitamin, they failed to exhibit the continuous and uniform growth response throughout an eight-week period that was characteristic of the animals used in Bourquin's work. This suggested a depletion of the bodily store of some factor or factors other than those generally recognized as necessary to mammalian nutrition. Whether Stiebel-

ing was dealing with more than one "new" factor in the two types of experiments outlined above cannot be ascertained from the data presented. It is interesting to note, however, that she obtained some evidence for the presence of two other "new" factors in the vitamin B complex, but her data relative thereto were so meager that she did not include them in her report. The question of the possible identity of Stiebeling's factor or factors with any of the other so-called "third" factors of the vitamin B complex mentioned in this review cannot at this time be settled.

"Third Factor" of Williams and Lewis

Our interest in the multiple nature of the vitamin B complex has extended over a period of many years. In 1921 and 1922, while working with Fay (15), we observed that the treatment of an adequate diet by different methods of heating (pressure cooking in acid and alkaline medium and baking in alkaline medium) destroyed the antineuritic factor in some cases and the rat growth factor in others, thus indicating that vitamin B was complex. In 1928 work was resumed on this problem of the multiple nature of the vitamin B complex in the hope that experimental evidence might be obtained for the presence of a factor or factors other than B and G. In 1930 Williams and Lewis (48) reported that after repeated extraction of yeast with increasing strengths of alcohol the yeast residue still retained a growth factor that supplemented the yeast extract, or the vitamin B (B_1) and G (B_2) fractions obtained therefrom, in producing normal growth of rats on a diet otherwise lacking in the vitamin B complex, a result that had not been obtained when the yeast extract, or its B and G fractions, constituted the sole source of B factors. We showed further that the yeast residue (Fraction R) was thermostable, and insoluble in water and alcohol. At that time we suggested the probable identity of our "third" factor and that reported by Hunt (24). Since that time work in our laboratory (Williams and Lewis, 49) has confirmed our earlier findings and demonstrated further that the yeast residue retains its potency in the "third" factor after prolonged extraction with acid alcohol, that the "third" factor is thermostable when autoclaved at 130°C for 24 hours in acid medium and when subjected to acid hydrolysis in 10 per cent hydrochloric acid at atmospheric pressure for 2 hours, that it is destroyed by heating in 10 per cent sodium hydroxide at atmospheric pressure for 2 hours, and that it is insoluble in hot water, hot alcohol, hot 10 per cent hydrochloric acid, and 50 per cent alcohol acidified to contain 1 per cent acetic acid. Still further work during the past few months (Rymer and Lewis, 43) has given addi-

tional confirmation of the presence of a third factor necessary for the normal growth of rats in the residue remaining from the aqueous-alcohol extraction of yeast, and has shown that alkaline autoclaving (5 hours; 15 lbs. pressure; pH 9.0) destroys the factor contained in the residue, whereas acid autoclaving (5 hours; 15 lbs. pressure; pH 5.3) has no effect upon it. Although our more recent results agree with those of Hunt and Wilder in showing the thermolability of our "third" factor in alkaline medium, the repeated demonstration of the insolubility of our factor in aqueous solution in contrast to the solubility of Hunt's factor makes it necessary to rescind our former view on the identity of the two factors and to reserve decision on this point for the present. The extreme insolubility of our "third" factor shows that it is not the same as the vitamin B_4 of Reader, although we may be dealing with a factor similar to that reported by Coward, Key, Morgan, and co-workers as present in casein. Further work must be done before the question of identity with any of the factors reported by other workers can be solved.

Other Reports on "Third" Factors of the Vitamin B Complex

In addition to the papers already mentioned, the literature contains several contributions of evidence for the existence of factors other than B and G in the vitamin B complex. The time at our disposal will not permit more than mere reference to them without any attempt to correlate the factors mentioned with the better defined factors previously reviewed. Among those that should be included are the factor necessary to prevent or cure heart block in pigeons (Carter and Drury, 3; Carter, 2); the factor whose absence leads to symptoms of intestinal stasis in rats and pigeons (Rosedale, 41, 42); the factor a deficiency of which causes muscular dystrophy in the guinea pig and the rabbit but not in the rat (Goetsch and Pappenheimer, 17); and the factor which possesses the power of counteracting the ill effects resulting from the ingestion of dried egg white by rats (Boas, 1; Fixsen, 16).

Guha and Drummond (19) have presented evidence which points to the multiple nature of vitamin B. The existence of two factors other than vitamins B and G, one in yeast and the other in milk, both of which are required for the normal growth of rats, has been claimed by Guha (18). Sure, Smith, and Kik (47) have suggested that vitamin G is composed of two components, the anti-dermatitis factor for which the G should be retained, and a growth factor for which they suggest the term vitamin F. It has been shown by Hartwell (22) that the factor which prevents kidney

abnormalities when high protein diets are fed to young rats is not the antineuritic vitamin B. Whether vitamin G or some other factor in the B complex is the "protective" agent is not shown by her work. This question has been under investigation in our laboratory (Race, Longwell, and Lewis, 33) recently.

Correlation of "Third" Factors

Thus, we see that our present knowledge concerning factors other than B and G in the vitamin B complex is in an extremely chaotic state. It seems hardly possible that there are as many factors as have been suggested; and yet the evidence at hand makes it impossible to do otherwise than to conclude that the vitamin B complex is composed of several definite entities and some that are less well defined. However, it seems altogether probable that further investigation will prove that some of the alleged factors are identical and that, instead of having as many components as are now claimed, the number will be reduced.

For the determination of the possible identity of certain so-called third factors of the vitamin B complex, two chief criteria have been used, namely their comparative requirements by the pigeon and by the rat and their relative thermostability. Unfortunately, the data in the literature on the latter point leave much to be desired, as there has been no uniformity in the conditions used by different investigators for the determination of the

TABLE I

"THIRD" FACTORS OF THE VITAMIN B COMPLEX

Name or tentative designation	First reported by	Required by		Behavior to alkali and heat
		Adult pigeon	Growing rat	
Vitamin B_3	Williams and Waterman	Yes	No	Labile
Vitamin B_4	Reader	No	Yes	Labile
Vitamin B_5*	Peters et al.	Yes	(?)	Very stable
Factor Y*	Chick et al.	(?)	Yes	Very stable
"Third" Factor	Coward et al.	(?)	Yes	Labile
"Third" Factor	Hunt	(?)	Yes	Labile†
"New" Nutritional Factor	Stiebeling	(?)	Yes	(?)
"Third" Factor	Williams and Lewis	(?)	Yes	Labile†

* Possible identity of these two factors suggested in this review.

† At first reported to be thermostable.

behavior of the different factors to heat. The importance of pH control in such comparative studies has been emphasized by Williams, Waterman, and Gurin (53), and by Chick and Copping (6). Certainly, unless the effect on two factors is studied under identical conditions of hydrogen ion concentration, temperature and degree of moisture, accurate conclusions as to the relative stability of the two factors cannot be made. Failure to observe these precautions has led to many contradictory statements in the literature as to the stability of the different factors of the vitamin B complex. However, since much of the evidence for the identity, or lack thereof, of the various so-called "third" factors has been based upon their stability or instability to heat, this information and an indication of whether the particular factor is required by the pigeon and by the rat are given in Table I.

Conclusion

In conclusion, we may well ask ourselves several questions. Which of these components of the vitamin B complex are required by man? Does he need all of these alleged factors claimed as vital for normal nutrition of the rat and of the pigeon? What deficiency diseases other than beriberi and pellagra may be a result of, or complicated by, a lack of these "newer" components of the vitamin B complex in the diet? Are beriberi and pellagra simple deficiency diseases due to an inadequate supply of a single specific vitamin in each case, or is a lack of two or more components of the vitamin B complex the basis of their etiology? Although the last question, particularly as regards pellagra, is receiving considerable attention, much additional information must be gathered before our knowledge concerning the complete rôle of the multiple components of the vitamin B complex in the maintenance of health and normal nutrition will be a closed chapter.

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